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BIOLOGICAL BULLETIN

CARBON DIOXIDE PRODUCTION IN RELATION TO REGENERATION IN PLANARIA DOROTOCEPHALA.[1]

HARRIET L. ROBBINS AND C. M. CHILD.

Numerous lines of evidence indicate that the body of *Planaria dorotocephala* consists physiologically of more than one individual or "zooid" after a certain limit of size is exceeded in the course of growth. The first or chief zooid of the series includes the region from the head to a level slightly posterior to the mouth, the level at which fission usually occurs, and the region posterior to this consists of one or more short zooids, the limits of which can be distinguished physiologically, but not morphologically (Child, '10, 11*c*, '15, chap. VI). The susceptibility of the body to a large number of chemical and physical agents in concentrations or intensities too high to permit acclimation or tolerance, decreases from the head posteriorly through the length of the first zooid, increases sharply at the level of fission and in long animals shows one or more rises posterior to that level (Child '13*b*). The process of regeneration also shows graded differences at different levels, corresponding to the differences in susceptibility (Child '11*b*).

In the papers referred to, as well as in many others evidence has been presented to that the susceptibility differences in general are in some degree an indicator of quantitative differences in metabolic condition, particularly in rate of oxidations, though susceptibility has never been regarded as an exact quantitative measure of oxidation. According to this point of view the gradations of susceptibility in the body of *Planaria dorotocephala* indicate that the rate of oxidation decreases gradually, at

[1] From the Hull Zoölogical Laboratory, University of Chicago.

least in the ectoderm and body wall, from the head to the level of fission and rises suddenly at the anterior end of the second zooid and of each further zooid, if such are present.

It has also been shown that in isolated pieces of the body of *Planaria*, particularly in those below a certain fraction of the body length, susceptibility is increased during the first few hours following section. This temporary increase of susceptibility, apparently associated with the stimulation of the pieces by section, is least in pieces nearest the head, and increases in successive pieces posteriorly to the level of fission, where it again decreases (Child, '14). In general the regions of highest susceptibility in the intact animal show the least increase in susceptibility in isolated pieces, and vice versa. Within twelve to twenty-four hours after section the temporary increase in susceptibility has disappeared and the susceptibility of each piece is about the same as, or slightly lower than before section. From twenty-four to forty-eight hours after section a gradual rise in susceptibility begins as the regulatory processes leading to the development of a new individual make themselves evident, and the susceptibility after regeneration is completed is much higher than that of the region of the body of the original animal which a given piece represents. This is true not only for the newly developed head and posterior end of the regenerated animal but for the whole body (Child, '15, Chap. V). In the light of various facts, these changes in susceptibility have been interpreted as indicating and in some way associated with changes in rate of oxidation. The present paper constitutes additional evidence for this conclusion. Carbon dioxide production was chosen instead of oxygen consumption as the subject of investigation first because investigation of the temporary changes, immediately following section involves the technical difficulty of preparing very large numbers of pieces within a very short time in order that oxygen consumption may be sufficient for determination in short periods, and second because work on oxygen consumption during the course of regeneration was already in progress in this laboratory. The colorimetric method was used since it is well adapted for obtaining comparative data with small amounts of material.

A few experiments similar to those recorded below were performed by Child from time to time during several years past, and these experiments, although they brought to light certain difficulties as regards technique and were too few in number to constitute conclusive evidence, indicated very clearly that the differences and changes in susceptibility following section and after regeneration are paralleled by differences and changes in rate of carbon dioxide production. A more extensive investigation of the subject by another person was desirable, and this was undertaken by Miss H. L. Robbins in candidacy for the degree of M.S. The data tabulated in the present paper are those obtained in this investigation.

The special technique for the different experiments is described in the following sections, but the general technique used in connection with the colorimetric method is very similar to that employed in the study of CO_2 production during starvation (Child, '19*a*). The weighing of the animals or pieces is the most difficult feature of the preparation, since the presence of superfluous water introduces an error in weight, and since weighing must be done as rapidly as possible to avoid injury or death from drying. After considerable practice, involving repeated weighings of the same lots and determination of the length of time which could be allowed without injury for drainage on filter paper and exposure to the air while weighing, a satisfactory method of procedure was developed, and this was followed in all the tabulated experiments. The container was first weighed and both container and weights were left on the balance pans: next weights equal to the estimated weight of the lot of worms or pieces were placed on the weight pan in order to reduce the time necessary for weighing the animals: the worms were then brought together at the tip of a funnel of well washed filter paper, drained for a certain length of time, transferred on a slightly vaselined scalpel blade to the container and weighed, each of these operations being performed in as nearly as possible the same length of time in each case. Equal or approximately equal weights of the lots to be compared were used (see tables). For the colorimetric estimations the Hynson Westcott and Dunning *H* ion outfit with phenolsulphonephthalein as indicator

was used. After weighing, the worms were returned at once to water and then transferred to pyrex tubes fused at one end and of the same diameter as the standard tubes. After washing twice in aqueous indicator solution of the same concentration as that in the standard tubes, each tube was filled with indicator solution to a 3-c.c. level previously marked. The tubes were then sealed without air bubbles by running in on the surface of the fluid about 1 c.c. of soft paraffin at a temperature just above melting point, the worms being kept at the bottom of the tube to avoid injury from change of temperature. Since leakage past the paraffin plug is difficult to avoid when changes of temperature occur and since it was found desirable to reduce the temperature slightly as a means of keeping the animals quiet (see below), the following method of providing for changes in volume of the fluid was used. A piece of closely fitting soft rubber tubing, previously coated with soft paraffin was drawn over the open end of the pyrex tube, leaving 2 to 3 cm. of the tubing beyond the end of the glass. Indicator solution was then added to fill both the glass tube above the paraffin plug and the rubber tube, and the latter is then closed by a screw clasp. This procedure makes impossible the entrance of air past the paraffin plug when the temperature is lowered. Instead of air a small amount of the indicator solution above the plug may be drawn below, but this occurs within the first five minutes or less of the experiment, and with the changes of temperature involved the amount of fluid passing the plug is negligible, so far as the results are concerned. The indicator solution between the paraffin plug and the rubber tubing is visible and serves as a control for the color change below the plug. Closure by means of the rubber tubing and clamp alone was found to be unsatisfactory because some of the worms creep into the rubber tubing, where they cannot be seen, and it is therefore impossible to determine whether motor activity is going on and whether all are in good condition. Moreover, pieces in the rubber tubing are often overlooked when the lots are removed from the experimental tube and the whole lot becomes valueless for further experiment, unless substitution for the lost pieces is made, but this is at best an undesirable procedure.

The *pH* at the beginning of the experiment was of course the same in all lots to be compared, but the starting point differed somewhat in different experiments, the extreme range being 7.8 to 7.95. Observations were made at least every half hour with few exceptions, but the tables, instead of recording all the *pH* readings, give the times required to reach an arbitrary end point, *Hp* 7.3 being selected as this end point. As each lot approached this point, observation was more or less continuous. A daylight lamp was used for all color comparisons.

A serious difficulty in the earlier experiments, particularly with the pieces after section, was the occurrence of motor activity, which of course increased CO_2 production and introduced a source of error. In these experiments as in earlier work, it was observed that pieces from regions near the head are much more likely to show apparently spontaneous motor activity during the first few hours after section, than pieces from the more posterior levels of the first zooid. After attempting in various ways to eliminate motor activity, it was found that decrease of a few degrees in temperature was usually effective for the length of time necessary. In all the experiments tabulated below the animals were kept at 21° to 22° C in the stocks and during preparation, but as soon as the tubes were sealed they were placed in water at a constant temperature of 18° in very dim diffuse daylight. Under these conditions motor activity occurred only rarely, but its occurrence was always noted in the record of the experiment. The procedure adopted in these experiments has been described at some length because in experimental work with animals of such small size it is extremely easy to go astray, if the various sources of error are not carefully controlled as far as possible.

The Stimulation of Pieces Following Section.

The temporary increase in susceptibility following section and its characteristic relation, both to size of piece and region of body (Child, 14, also p. 104 above) are so clearly shown by the susceptibility method that they are often used as laboratory experiments. Because they are temporary and apparently excitatory in character and so definitely related to size of piece

and region of body these changes in susceptibility are of special interest in connection with the question of the relation between susceptibility and metabolism. If changes in the rate of fundamental metabolism or of certain fundamental reactions are found to parallel these changes in susceptibility, it is evident that the susceptibility method, when properly used, is a rather delicate indicator of at least certain aspects of metabolic condition.

In the earlier experiments of Child, as well as in the preliminary work of Robbins, it was found that the changes and differences in CO_2 production following section appeared more clearly in animals which had been starved for a few days before experiment, than in those which had been more recently fed, although in the former the total CO_2 production and oxygen consumption are less than in the latter (Child, '19*a*, Hyman, '19*b*). It has been pointed out elsewhere (Child, '19*b*, '19*c* and various earlier papers), that the susceptibility method as used in these experiments gives information primarily concerning conditions in ectoderm and body wall, though with certain precautions it may be used to show differences in condition in the alimentary tract of *Planaria* and other forms. The susceptibility data, as well as other facts, indicate that the changes following section are at least in large measure confined to ectoderm and body wall, the alimentary tract not being affected to any great degree. The CO_2 production of the alimentary tract in fed animals constitutes, however, a large proportion of the total CO_2 production, moreover, the volume of the alimentary tract as compared with that of other organs is greater and a larger amount of food and reserves is usually present in regions near the mouth than in regions near the head, therefore it is desirable to decrease this alimentary CO_2 production as far as possible, in order that changes in other parts of the body may appear more clearly. It has been shown (Child, '19*a*, Hyman, '19*b*) that a rapid decrease in both CO_2 production and oxygen consumption occurs in *Planaria dorotocephala* during the first few days of starvation and that an increase in both follows so rapidly after even a single feeding that it cannot be due to oxidation of the food following assimilation, but must be due to stimulation of the alimentary tract by the food. Allen ('19) has recently recorded the occurrence of similar

changes in oxygen consumption in two other species of *Planaria* during the early stages of starvation. It is evident that the rapid decrease in CO_2 production and oxygen consumption during the early stages of starvation is due in large measure, if not wholly, to the decrease in functional activity of the alimentary tract in the absence of food newly ingested. In the light of all these facts the reason for the use in these experiments of animals which have been starved a few days is evident. The length of the period without food is given in each experiment: in no case is it long enough to produce any marked reduction in size or other changes except those in the alimentary tract.

Since the purpose of these experiments is to determine whether differences and changes in susceptibility following section are paralleled by differences and changes in CO_2 production, the size of animals and pieces used and the regions of body included are those which show the most definite and characteristic differences and changes in susceptibility. The experimental material for the data presented in Table I. was prepared as follows: animals sixteen to eighteen mm. were selected from well fed laboratory stock, those which had recently undergone fission being excluded, were kept without food for several days (see Table I.) and were then cut into pieces as indicated in Fig. 1, piece *C* being cut so that the greater part of the pharynx is separated from its attachment and is extracted from the pharyngeal pouch, *i.e.*, piece *C* contains a part of the pharyngeal pouch, but no portion of the pharynx. Pieces *A* and *C* were used in the experiments as representing respectively the most anterior and the most posterior portion of the first or chief member or zooid in animals of this size (Child, '11*b*). In the intact animal the susceptibility of the region corresponding to piece *A* is very much greater than that of the region corresponding to piece *C*. Immediately after section the susceptibility of piece *A* shows either a slight increase or no marked change, while the susceptibility of *C* is increased to such a degree that it is equal to or even greater than that of *A* (Child, '14). The region *B* between *A* and *C* is intermediate both as regards original susceptibility and the changes following section and is not used in these experiments. The increase in susceptibility following section, which is most marked in the *C*-piece is

temporary and after six to eight hours is in course of disappearance and sooner or later (12 to 24 hours under ordinary conditions) the susceptibility of the piece becomes about the same as or a little less than that of the corresponding regions in the intact animal. These temporary changes in susceptibility indicate that the pieces have been stimulated by section, anterior pieces least, posterior pieces most, and that the condition of excitation disappears after a few hours. It has been suggested elsewhere (Child, '14) that the difference in the degree of stimulation in anterior and posterior pieces results from the greater dependence of the more posterior regions upon impulses from regions anterior to them, so that when the paths of these impulses are cut the condition of the more posterior levels is more affected than that of the anterior levels. This is in agreement with the observations of various investigators concerning the difference in intensity of reaction in many animals between levels anterior and those posterior to a transverse cut.

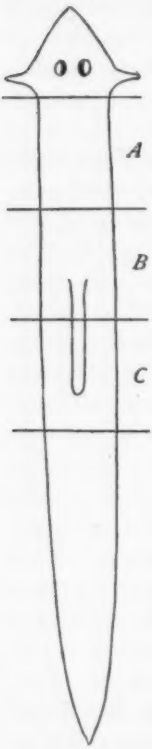

FIG. 1.

The region of the body posterior to *C* in Fig. 1 is not used in the tabulated experiments because it consists of one or more zooids indicated by differences in physiological condition (Child, '10, '11*c*). Since the number of these posterior zooids differs in different animals and no morphological boundaries are visible, pieces from the same levels of this region in different animals are not always strictly comparable physiologically and therefore merely complicate the experimental data.

Each experiment on CO_2 production in the pieces *A* and *C* consists essentially in determining the rate of change in *pH*, once as soon as possible after section and again several hours later, of one lot of *A*-pieces and one of *C*-pieces, consisting of thirty to fifty pieces each and of as nearly as possible equal weight, in equal volumes of indicator solution at a constant temperature

of 18° C. As soon as *pH* 7.3 is reached in the first determination, the tubes are opened, the pieces are returned to water and left undisturbed for the desired number of hours, *i.e.*, at least long enough as determined by the susceptibility method for disappearance in large measure of the temporary changes following section. At the end of this time the rate of *pH* change is again determined for each lot under as nearly as possible the same experimental conditions as the first determination.

Fifteen such experiments were performed before the technique used in the experiments recorded in Table I. was fully worked out. In eight of these fifteen experiments the *A*-pieces showed marked motor activity and a more rapid change in *pH* than the *C*-pieces immediately after section, in three there was either death of some of the pieces from drying or an error in weighing, and in four none of these sources of error was involved and the result were similar to those in Table I.

Table I. gives the data for thirteen experiments, in all of which the technique was as nearly as possible the same. The only feature of the table which requires explanation is the column "Time after section." The times given in this column are the times of sealing of the tubes containing the animals in indicator solution. The first time for each lot, "Immed.," *i.e.*, immediately, means simply that the *pH* determination was begun as soon as possible after the pieces were sectioned. Since it requires a half hour or more to cut lots consisting of forty to fifty *A* and *C*-pieces, the time between section and sealing may be as much as an hour for the pieces cut first and only a few minutes for those cut latest.

In eleven of the thirteen experiments recorded, 84 per cent., the CO_2 production of the *C*-pieces immediately after section is about equal to, or greater than that of the *A*-pieces. As regards the other two experiments, Nos. 7 and 8, in No. 7 three *C*-pieces died during the first determination, and in No. 8, motor activity occurred in the *A*-pieces, perhaps because of a slight rise in temperature of the water in which the tubes were kept. The second *pH* determination, begun at various lengths of time ranging from nine to forty-two hours after section, shows in every case a lower rate of CO_2 production in *C* than in *A*.

TABLE I.

A COMPARISON OF ANTERIOR AND POSTERIOR PIECES IMMEDIATELY AFTER SECTION AND A NUMBER OF HOURS LATER.

Expt. No.	No. of Pieces.	Wt. in Grams.	No. of Days Without Food.	Temperature in Degrees C.	pH Starting Point.	Time After Section in Hrs. and Min.	Time in Hrs and Min. to Reach pH 7.3.
1	A 37	0.023	11	17.5	7.8	immed.	5:40
						22:30	5:40
	C 31	0.0231	11	17.5	7.8	immed.	5:25
						22:30	5:55
2	A 49	0.0255	24	18	7.9	immed.	6:25
						9:00	6:30
	C 45	0.0257	24	18	7.9	immed.	6:15
						9:00	6:45
3	A 56	0.0245	21	18	7.82	immed.	5:18
						19:40	6:05
	C 48	0.0243	21	18	7.82	immed.	5:05
						19:40	6:23
4	A 20	0.0182	8	18	7.83	immed.	6:10
						18:40	6:22
	C 23	0.0185	8	18	7.83	immed.	6:00
						18:40	6:45
5	A 51	0.0208	9	18	7.83	immed.	5:30
						20:15	5:35
	C 44	0.0207	9	18	7.83	immed.	5:05
						20:15	5:55
6	A 37	0.0174	12	18	7.83	immed.	6:30
						18:00	6:35
	C 42	0.0176	12	18	7.83	immed.	6:10
						18:00	6:45
7[1]	A 37	0.0143	13	18	7.83	immed.	7:20
						21:30	7:45
	C 37	0.0142	13	18	7.83	immed.	7:35
						21:30	8:00
8[2]	A 40	0.0216	7	18	7.83	immed.	4:50
						17:45	5:05
	C 41	0.0213	7	18	7.83	immed.	4:55
						17:45	5:10
9[2]	A 36	0.0238	8	18	7.83	immed.	4:50
				18.5		19:00	4:38
	C 36	0.0239	8	18	7.83	immed.	4:35
				18.5		19:00	4:50
10	A 37	0.0242	9	18	7.83	immed.	4:40
						22:00	4:55
	C 38	0.0241	9	18	7.83	immed.	4:15
						22:00	5:15
11	A 44	0.0242	7	18	7.95	immed.	5:30
						19:00	5:35
	C 45	0.024	7	18	7.95	immed.	5:10
						19:00	5:50
12	A 44	0.0306	8	18	7.95	immed.	4:45
						42:00	4:40
	C 50	0.0305	8	18	7.95	immed.	4:15
						42:00	4:50
13	A 29	0.021	13	18	7.8	immed.	4:05
						25:00	4:10
	C 21	0.0208	13	18	7.8	immed.	3:50
						25:00	4:25

As regards the two records for the same lot, the *A*-pieces show in general a slightly lower CO_2 production in the second period than in the first, or in some cases about the same. Experiment 9, however, shows a slightly higher rate in *A* during the second period, this being due probably to the slightly higher water temperature during the second period. In the *C*-pieces the rate is distinctly lower, in many cases much lower in the second period than in the first. Differences in time of five minutes between the records of two lots made at the same time mean only that the two were barely distinguishable as different. Differences of this mangitude between the two records of the same lot can mean no more than that the rate is essentially the same in the two periods. Differences of ten minutes or more are however, unquestionably significant and differences of much greater magnitude appear in the table.

The table shows then that immediately after section there is in some cases a slight temporary increase in CO_2 production in the *A*-pieces and a very marked increase in the *C*-pieces, that is to say the differences and changes in CO_2 production following section are in general parallel to the differences and changes in susceptibility. The objection may be raised that the differences in CO_2 production are less than would be expected from the stimulation by section, but when it is remembered that there is no reason to believe that the alimentary tract shares in this stimulation except locally at the cut surface and that the pieces do not undergo motor activity during the determination this objection has little weight. As a matter of fact the close parallelism between the data on CO_2 production and those on susceptibility indicates that even as regards the temporary changes following section of pieces, susceptibility is in some degree

[1] During the first determination in No. 7 three *C*-pieces died. When the determination was repeated the following day, two *C*-pieces, which had been cut with the others but not used before, were added to lot *C* and one piece was removed from lot *A* to make weights as nearly as possible equal. Only two extra worms had been sectioned, therefore three pieces could not be added to take the place of those dead.

[2] Slight motor activity occurred in the *A*-pieces during the first determination.

[3] Here the temperature was half a degree higher in the second determination than in the first, and the *A*-pieces show a slightly increased rate in the second determination. Footnotes for Table 1.

a measure of physiological and particularly of respiratory condition.

Carbon Dioxide Production after Regeneration.

In these experiments stocks of pieces of the size of *A*, *B*, *C*, Fig. 1, were cut from animals 16–18 mm. and allowed to undergo regeneration until the development of the new individuals was essentially complete, usually about two weeks. From these stocks the experimental lots were selected. These consisted only of normal individuals, or in some cases where the number of normal animals in the regenerated stock was not sufficient, mostly of normal with a few teratophthalmic individuals (Child, '11*a*) added. Since the pieces undergoing regulatory development cannot feed until they attain an advanced stage, the animals representing the condition before regulation, with which the regenerated individuals are to be compared, are kept without food for the same length of time as the pieces undergoing regeneration. Usually a stock of intact worms of the same size, 16–18 mm., as those from which the pieces were taken is isolated at

Table II.

A Comparison of Small Animals Regenerated from Pieces with Large Animals that Have Not Recently Undergone Regeneration or Fission, of the same size as those from which the pieces were taken. Both Unfed. Determinations of pH at + 18° C.

Expt. No.	No. of Worms.	Wt. in Grams.	Period of Regeneration or Starvation in Days.	pH Starting Point.	Time in Hrs. and Min. to Reach pH 7.3.
1	3 large	0.0114	10	7.89	10:25
	16 regen.	0.0113	10	7.89	9:00
2	4 large	0.0163	11	7.89	8:55
	22 regen.	0.016	11	7.89	6:45
3	4 large	0.0174	10	7.89	8:00
	27 regen.	0.0176	10	7.89	6:15
4	4 large	0.0117	14	7.9	14:35
	19 regen.	0.0115	14	7.9	12:35
5	5 large	0.0205	14	7.9	9:35
	34 regen.	0.0204	14	7.9	6:50
6	3 large	0.0101	21	7.8	10:00
	26 regen.	0.099	7 starved 14 regen.	7.8	7:45
7	3 large	0.0109	20	7.8	8:30
	19 regen.	0.011	7 starved 13 regen.	7.8	7:50
8	3 large	0.0166	10	7.8	6:30
	28 regen.	0.0164	10	7.8	3:50

the same time the pieces are cut. This stock merely undergoes a slight degree of starvation, while the pieces undergo starvation for the same period and in addition the regulatory changes. This is the procedure in experiments 1–5 and 8 in Table II., but in experiments 6 and 7 the stock from which both pieces and whole animals were obtained was starved seven days before the pieces were cut.

Each experiment in Table II., includes one lot of worms about 5 mm. in length which have developed from pieces cut ten to fourteen days earlier ("regen." in table), and one lot of as nearly as possible the same weight of worms 16–18 mm. in length, the same size and from the same general stock as the worms from which the pieces were cut, and kept without food for the same length of time ("large" in table). In all cases the *pH* determinations are made before feeding is resumed.

Examination of the last column of the table shows that the rate of CO_2 production is much higher in the small regenerated, than in the large old animals, *i.e.*, the regulatory processes have been accompanied by an increase in rate of CO_2 production. Moreover the rate is in general higher in the regenerated animals

TABLE III.

A COMPARISON OF SMALL ANIMALS REGENERATED FROM PIECES WITH LARGE ANIMALS OF THE SAME SIZE AS THOSE FROM WHICH THE PIECES WERE TAKEN. Fed three times. Determinations of pH at 18° C.

Expt. No.	No. of Worms.	Wt. in Grams.	pH Starting Point.	Time in Hours and Minutes to Reach pH 7.3.
1	3 large	0.0127	7.82	5:00
	10 regen.	0.0125	7.82	4:30
2	4 large	0.0193	7.82	3:45
	15 regen.	0.0191	7.82	2:55
3	4 large	0.0154	7.82	4:50
	13 regen.	0.0155	7.82	3:35
4	4 large	0.0135	7.9	7:15
	18 regen.	0.0134	7.9	5:50
5	5 large	0.0233	7.9	5:05
	28 regen.	0.023	7.9	3:45
6	2 large heads off	0.0155	7.8	8:15
	18 regen.	0.0113	7.8	4:25

than in the pieces of Table I. although the period without food is in most cases longer in the latter than in the former and CO_2 production decreases during the early stages of starvation.

Table III. records experiments similar to those of Table II.

except for the fact that both lots were fed with beef liver three times before weighing and *pH* determination, the first two feedings being on successive days, the third after an interval of one day. In the first five experiments of Table III. worms from the first five experiments of Table II. were used, but in smaller numbers because of the increased weights after feeding, particularly in the regenerated animals. In Table III., as in Table II. the "large" animals are those which have not undergone regeneration and represent as nearly as possible the animals from which the pieces were taken, and the "regenerated" animals are those which have developed from the pieces. Table III. agrees with earlier work (Child, '19*a*) in showing that the rate of change in *pH* is increased in all animals by feeding after a period of starvation, but it also shows that the difference in rate between the regenerated and the large animals persists after feeding. Here again the data on CO_2 production agree with the results of the susceptibility method (Child, '15, Chap. IV.). Data on oxygen consumption recently published by Allen ('19) and by Hyman ('19*b*) also agree with these results.

Additional Data.

Table IV. includes a number of miscellaneous experiments of some interest.

In the course of regulatory development an outgrowth of new tissue occurs at anterior and posterior ends of each piece. All the facts indicate that this tissue, which forms the new head and posterior end, is more or less embryonic in character when it arises and possesses, at least at first, a higher rate of metabolism than the remainder of the piece. In order to determine whether the higher rate of CO_2 production in regenerated animals is due solely to the more intense activity of this new tissue or whether the rate is also increased in other parts, the new heads and posterior ends were removed from regenerated animals leaving only the so-called old or less extremely altered tissue of the middle regions. Lots of such pieces were then compared with lots of equal weight of freshly cut *A*-pieces (Fig. 1) and of animals 16–18 mm. like those from which the pieces were taken, the heads being removed from these large animals in order to

make them more nearly comparable in condition with the *A*-pieces and the headless regenerated animals.

Experiment 1 of Table IV. includes one lot of each of the three groups and it is seen that the "old" parts of the regenerated

TABLE IV.

MISCELLANEOUS DATA.

Regenerated animals from which anterior and posterior new tissue has been removed, compared with *A*-pieces and with large headless animals: whole regenerated animals compared with *A*-pieces and with growing worms of same size from stock. Fed or unfed. Determinations of pH at 18° C.; pH at beginning of experiment 7.8.

Expt. No.	No. and Condition of Animals.	Wt. in Grams.	Nutrition.	Time in Hours and Minutes to Reach pH 7.3.
1	3 large, heads off.	0.093	Starved 19 days.	8:25
	12 *A*-pieces.	0.094	Starved 19 days.	7:15
	11 regen. new tissue off.	0.095	Starved 18 days.	6:25
	9 *A*-pieces.	0.08	Fed three times.	8:15
2	22 regen. new tissue off.	0.082	Fed three times.	7:30
	3 large.	0.0111	Fed three times.	7:35
3	15 regen. new tissue off.	0.0108	Fed three times.	6:05
4	22 *A*-pieces.	0.0167	Starved 10 days.	4:10
	28 regen.	0.0164	Starved 10 days.	3:50
5	11 small.	0.0096	Fed three times.	6:25
	18 regen.	0.0095	Fed three times.	5:25

animals show a higher rate of change than the *A*-pieces, while the headless large animals show the lowest rate of all.

In Experiment 2 the "old" parts of regenerated animals are compared with *A*-pieces, both lots being fed three times before sectioning. The result is the same as in experiment 1, the rate being distinctly higher in the parts of regenerated animals than in the *A*-pieces.

In experiment 3 the "old" parts of regenerated animals are compared with large old animals from which the heads have not been removed, both lots being fed three times after a starvation period of about two weeks. The result is the same as in Experiment 1, the parts of regenerated animals showing the higher rate.

Experiment 4 is a comparison of *A*-pieces with entire regenerated animals, *i.e.*, including the new heads and posterior

ends. Here the difference in rate is proportionally about the same as in Experiment 2, but somewhat less than in Experiment 1.

In Experiment 5 regenerated animals are compared with stock animals of slightly larger size (the smallest in the stock at the time) which were kept without food while the pieces were regenerating, both lots being fed three times before the experiment, and both consisting of entire animals. Here again the regenerated animals show a higher rate of CO_2 production than the slightly larger stock animals.

A few other experiments performed by one of us and in some cases also by students in the laboratory, are briefly mentioned here without tabulation of the data. It has been found, for example, that the degree of increase in both susceptibility and CO_2 production occurring in regeneration depends upon the degree of reorganization which occurs. Consequently the smaller the piece in relation to the size of the body from which it is taken, the greater the amount of increase in susceptibility and CO_2. Similarly in natural fission the posterior piece is not only smaller than the anterior but develops a new head at the anterior end and a prepharyngeal and pharyngeal region by reorganization and redifferentiation within the piece, while the anterior fission piece develops merely a new posterior end. In the animal developed from the posterior piece susceptibility and CO_2 production show a marked increase while in the anterior animal the only marked change in susceptibility is a slight increase in the posterior region, where reorganization and growth have occurred and the increase in CO_2 production is either slight or inappreciable. Allen ('19) has recently recorded somewhat similar results as regards oxygen consumption, an increase occurring in the posterior, but not in the anterior product of fission.

It has also been determined by one of us that susceptibility to lack of oxygen increases during the development of a new individual from a piece, the susceptibility of the new individual about two weeks after section of the piece, being distinctly higher than that of well fed animals of the same size and about the same as, or slightly than that of animals of the same size, kept without food for the same length of time as the regenerating

pieces. In other words, the new individuals developed from pieces show a susceptibility to lack of oxygen equal to or greater than that of much smaller younger animals than those from which the pieces were taken. These observations concern primarily the susceptibility of ectoderm and body wall.

Conclusion.

These experiments constitute a new test of the validity of the susceptibility method as a rough comparative means of determining physiological or metabolic condition and at every point the differences and changes in susceptibility, as determined by KNC and in many cases by various other agents also, are paralleled by differences and changes in rate of CO_2 production. Even the temporary stimulation of the pieces after section, which is slight or absent in the *A*-pieces and very marked in the *C*-pieces, appears in the data on CO_2 production and the increase in rate, at least of respiratory metabolism associated with regulation, is evident in the marked increase in rate of CO_2 production even in the "old" parts of the regenerated animal. This work may perhaps be regarded as in some respects the most delicate test of the relation between susceptibility and respiration which has been made up to the present.

As has been repeatedly stated, the susceptibility method is not an exact quantitative method, but a rather crude means of indicating differences of some sort in physiological condition and the differential susceptibility of different regions of the same individual affords a means of modifying and controlling various developmental and other processes. The facts at hand concerning susceptibility, *e.g.*, the lack of specificity, the close relation between susceptibility and physiological activity in development, growth and function as well as the positive evidence already obtained concerning the parallelism between susceptibility, oxygen consumption and CO_2 production indicate very clearly that a more or less definite relation exists between the susceptibility of living protoplasm, to at least many external agents and conditions within certain ranges of concentration or intensity, and the rate or intensity of certain fundamental physiological processes, particularly those which liberate energy.

This is all that is meant when susceptibility is interpreted in terms of metabolism or oxidation and the exact nature, degree and extent of this relation of course remains to be determined. This interpretation does not involve the assumption that susceptibility must always be parallel or even proportional to total oxidation or even to total oxygen consumption or CO_2 production.

The relation between susceptibility and oxidation is undoubtedly indirect in at least most cases and it is conceivable that susceptibility may be related only or primarily to certain oxidative reactions or to conditions associated with them. Moreover, it is certain that in many cases susceptibility as determined by death and disintegration is dependent primarily upon conditions or reactions in particular regions of the body, *e.g.*, in ciliate infusoria the ectoplasm, in *Planaria* the ectoderm and body-wall. Moreover, as many investigators have pointed out, it is by no means certain that oxygen consumption and CO_2 production are exact quantitative measures of oxidation at any given time. It is to be expected that susceptibility will not always be proportional to total oxygen consumption or CO_2 production, but even then susceptibility may prove in the long run to be a better indicator or comparative measure of physiological condition than the respiratory data.

From what has been said above and in earlier papers (*e.g.*, Child, '19*c*) it is evident that the criticisms of the susceptibility method recently advanced by Lund ('18*a*, *b*) and Allen ('18, '19) need no discussion here, since they are largely beside the point and result from failure to grasp the conception of susceptibility, which has developed from many different lines of investigation, not from one alone. Even if we grant the correctness of certain of their conclusions from experimental data which are or appear at present to be in conflict with conclusions reached in this laboratory (Child, '19*a*, *b*, *c*, Hyman, '19*a*, *b*) on the basis of more extensive investigation, with more satisfactory technique and several different methods instead of one they do not constitute adequate grounds for denying the physiological significance of susceptibility, but rather merely a starting point for the further analysis of the particular cases in question.

As regards the real significance of susceptibility, it makes

little difference whether or not it shall be found to run exactly parallel to the other indices of total respiration in any particular case. It cannot, however be denied that a wide range of facts determined by many different lines of investigation do indicate clearly the existence of a more or less definite relation between susceptibility and oxidation in at least many cases, and it is of interest to determine range, degree and nature of this relation. The present paper like several others which have recently appeared from this laboratory is a contribution to this problem, but it must be remembered that data such as these are not the only criteria of the physiological significance of susceptibility and its relation to the energy-liberating reactions in the metabolic complex.

SUMMARY.

1. The colorimetric estimation of CO_2 production shows that the changes in CO_2 production following section in pieces of the body of *Planaria dorotocephala* run parallel with changes in susceptibility. Immediately following section CO_2 production is markedly increased in pieces cut from near the mouth region, while in pieces from regions near the head it is only slightly if at all increased. These changes are temporary excitations following section and disappear after a number of hours.

2. The development of a new individual from a piece is accompanied by a very considerable increase in CO_2 production which involves not only the new outgrowths at the two ends of the new animal but the "old" parts as well. This increase in CO_2 production is found both before and after feeding is resumed following the development of the piece. In these respects also the changes in CO_2 production parallel changes in susceptibility, both series of data indicating that the animal developing from an isolated piece becomes in the course of this development, physiologically younger than the animal from which the piece originated.

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SUSCEPTIBLE AND RESISTANT PHASES OF THE DIVIDING SEA-URCHIN EGG WHEN SUBJECTED TO VARIOUS CONCENTRATIONS OF LIPOID-SOLUBLE SUBSTANCES, ESPECIALLY THE HIGHER ALCOHOLS.

FRANCIS MARSH BALDWIN.

INTRODUCTION.

That dividing *Arbacia* eggs show periods of varying susceptibility and resistance when exposed to chemical substances and to various physical conditions has been proved by numerous investigations. When eggs, from a single fertilized lot were placed at regular successive intervals after fertilization in cyanide-containing sea-water ($m/100$ to $m/200$), Lyon[2] found that they were highly resistant to poisoning fifteen or twenty minutes after fertilization, while eggs exposed to the same solution at the time of cytoplasmic division were promptly killed. Later, after the first division had been completed, the resistance to poisoning again returned, followed by a second susceptible period at the second cleavage. Loeb[3] later noted that the unfertilized eggs show greater resistance to cyanide poisoning than the fertilized eggs, and Mathews[4] indicated that in dividing eggs, the period of maximum susceptibility is "immediately before and during segmentation," and that just after segmentation the egg becomes relatively highly resistant. Similar results were obtained by Spaulding[5] in experiments with weak solutions of ether (1/64 per cent. in sea-water). The period of high resistance continued up to the beginning of the first cleavage, and then fell during cleavage to zero, with a sharp rise immediately afterwards. There was a short period of susceptibility immediately following

[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Department of Zoölogy, Iowa State College, Ames, Iowa.

[2] E. P. Lyon, *Amer. Jour. Physiol.*, 1902, Vol. 7, p. 56.

[3] J. Loeb, *Biochem. Zeitschr.*, 1906, Vol. 1, p. 200.

[4] A. P. Mathews, BIOL. BULL., 1906, Vol. 11, p. 137.

[5] E. G. Spaulding, BIOL. BULL., 1904, Vol. 6, p. 224.

fertilization. He found also in acid and salt solutions (pure isotonic KCL and NaCL) a similar but less clearly defined rhythm of susceptibility. Eggs subjected to heat, electrical stimulation and hypertonic sea-water behave in a similar manner. Thus, Lyon,[1] observed that the eggs were most resistant to heat at a time previous to the first cleavage, and were most readily injured at the time of division. A. R. Moore[2] finds that the resistance to hypertonic sea-water is least "immediately before and during each cytoplasmic division, and that the maximal resistance is shown 35 to 45 minutes after fertilization and just after each division." More recently Lillie[3] (1916), has made an extensive study of the rhythmical changes in the resistance of the dividing sea-urchin egg to hypotonic sea-water, and has discussed the physiological significance of this rhythm. His experiments show clearly that at or about the time of formation of the cleavage furrow, a marked decline takes place in the resistance of the egg to hypotony, and cytolysis is then rapid and complete. After the cleavage furrow is fully formed the original resistance returns. A similar reversible decline of resistance takes place at the second and third cleavage, and is probably general for mitotic cell-division. The minimum of resistance is found during the formation of the furrow. Both the decline and the return of resistance are rapid, the greater part of each phase occupying four to five minutes. Some increase of susceptibility is apparent ten or twelve minutes before the first appearance of the furrow. Similar observations have been made by Herlant[4] in the egg of *Paracentrotus lividus*.

From such experiments it appears that the resistance of the eggs to a variety of injurious agencies is least at the time when they are undergoing rapid change of form. To account for these rhythmical changes in the physiological state of the egg, Lillie[5] (1909) puts forward the hypothesis that they are essentially the result of variations in the physical condition, especially the permeability, of the surface-film of plasma-membrane, the latter

[1] E. P. Lyon, *Amer. Jour. Physiol.*, 1904, Vol. 11, p. 52.
[2] A. R. Moore, BIOL. BULL., 1915, Vol. 28, p. 257.
[3] R. S. Lillie, *Jour. Exper. Zoöl.*, 1916, Vol. 21, No. 3, p. 401.
[4] M. Herlant, *Comptes rendus d. l. Societe d. Biologie*, 1918, Vol. 81, p. 151.
[5] R. S. Lillie, BIOL. BULL., 1909, Vol. 17, p. 207.

undergoing a reversible increase in permeability at the time of cleavage. If a rhythm of alternate increase and decrease of permeability accompanies the rhythm of the mitotic process, it seems logical to infer that the entrance of solutes into the cell would occur most readily when there is a loss of semi-permeability. Accompanying this change would be a decrease of the electrical surface-polarization, and this in turn probably would alter the metabolic processes, especially oxidations within the cell. Cell metabolism then is inseparably bound up with cell-permeability; and the plasma-membrane, or semi-permeable surface-layer is something more than a haptogen membrane (to which it has frequently been compared). In discussing this subject in a later paper, Lillie[1] makes it especially clear that this "general characteristic of semi-permeability (the all-essential insulating and diffusing-preventing property) is not merely the result of a special chemical composition and structural density, such as determine the semi-permeability of a precipitation-membrane, but is inseparable from the living condition, *i.e.*, is actively maintained by a continual process of metabolism. The proof of this is that death—the cessation of metabolism—however caused, is invariably followed by a loss of semi-permeability, *i.e.*, the normal state of the membrane then ceases to be maintained and the unhindered processes of diffusion lead to the disintegration of the cell. Hence destruction of the surface-layer by artificial means—cytolytic substances, heat, extensive mechanical injury—is quickly fatal to all cells."

In the experiments about to be described, I have studied the behavior of fertilized Arbacia eggs when subjected for definite brief lengths of time to various concentrations of some of the higher alcohols—anyl, hexyl, heptyl, octyl and capryl—at different periods of the cell-division cycle. This work was undertaken at the Marine Biological Laboratory, at Woods Hole, Mass., during the past summer at the suggestion of Professor Ralph Lillie, to whom the writer expresses his hearty thanks for many kind suggestions and directions during its prosecution.

[1] R. S. Lillie, *Amer. Journ. Physiol.*, 1918, Vol. 45, No. 4, p. 406.

Experimentation.

In order to procure a sufficient number of eggs for each series of experiments, between one and two dozen large females were opened, and their eggs collected into finger bowls. By successive washing and settling, a uniform mass of mature eggs was obtained, which could be inseminated and divided into two parts; one to be used for the control, and the other for the experiments. It was found early in the work that the success of the experiments depended upon having batches of eggs which were sufficiently mature and uniform, so that all eggs reached successive stages in their development at practically the same time. It was also found that great exactness in the time-relations of the operations was absolutely essential, and that any variation once entered upon was sufficient to make the results worthless from a comparative standpoint. Usually two series of experiments were started in a day; one in the morning to be carried over to the gastrula stage by the following morning, and one in the afternoon, to be examined the following afternoon. After extended preliminary experimentation, it was found convenient, in any one series, to keep the time of exposure constant and to vary the concentration of substance used, although in a considerable number of experiments the opposite procedure was adopted, *i.e.*, the time was varied and the concentration kept constant.

Practically the same procedure was observed throughout the entire experimentation. At each of the successive intervals after fertilization, usually ten minute intervals, about one half of medicine pipette containing a suspension of the inseminated eggs was placed in a small corked Erlenmeyer flask, containing 50 c.c. of the solution of the alcohol in sea-water, and allowed to remain for the time of exposure chosen (usually five minutes). After the given time had nearly elapsed, the excess liquid was poured off, and the eggs with a little of the liquid were placed in a watch glass and the immediate results of the treatment were observed under the low power of the microscope. At the termination of the time of exposure, the watch glass containing the eggs was carefully immersed in a large volume of sea-water in a finger bowl and the water was changed several times to rid it of the excess substance. Finally the eggs were very carefully

washed with a stream of water from the medicine-dropper, and set aside to undergo development. The proportion proceeding with development to the free-swimming larval stage was subsequently determined. It was found that the estimate of the proportion surviving to the blastula stage was more readily and exactly made, if the watch glass containing the eggs was removed from the bowl of sea-water just before the free-swimming larval stage was reached. Thus all survivors could be confined within a small volume, and the count or estimate easily made. As a rule, the experiments were carried only up to about the time of second cleavage; since the evidence indicates that the same variation of susceptibility occurs in each cell division cycle; moreover divergencies between the different eggs in any lot become more pronounced as time elapses, and it is important that all eggs of a lot should be in the same physiological state at the time of treatment.

At first several preliminary experiments were necessary in order to determine the most suitable range of concentrations to be used, since the time of exposures determined upon were brief, the longest being ten minutes; in some cases of exposures only three minutes were used. In this connection, the tables given by Lillie[1] in his paper on the action of various anæsthetics in suppressing cell-division in sea-urchin eggs, were exceedingly helpful. For i-Amyl[2] alcohol, he finds 0.45 to 0.4 vol. per cent. a favorable anæsthetic concentration for eggs subjected for two and one half hours, while 0.5 vol. per cent. and above are somewhat rapidly toxic. For Capryl[3] alcohol he finds the anæsthetizing concentrations to range between 0.012 and 0.02, and notes that even in sub-anæsthetic concentrations this alcohol exhibits a relatively high specific toxicity. With the help of these data, and also Führer's[4] observations showing that in a series of monohydric aliphatic alcohols each member of the group is from three to four times as effective (for equimolecular concentrations)[5] as its immediate predecessor, it became a compara-

[1] R. S. Lillie, *Journ. Biolog. Chem.*, 1914, Vol. 17, No. 2, pp. 129–139.

[2] Cf. reference just cited; Table VIII., p. 135.

[3] Cf. reference just cited; Table IX., p. 137.

[4] H. Führer, *Arch. f. exp. Path. u. Pharm.*, 1904, LII., p. 69.

[5] Capryl alcohol used in exposures of five minutes seemed not to obey this general rule, since in practically all experiments it was used in concentrations nearly three times its computed strength. (See p. 137.)

tively easy matter to approximate the most suitable concentration of each alcohol after the first had been determined.

Amyl Alcohol.

Summarizing briefly the results of preliminary observations it was found that the most satisfactory concentration of i-Amyl alcohol when used with exposures of three to eight minutes, was between 0.7 and 0.9 vol. per cent. Solutions of this strength are sufficiently toxic to prevent many but not all of the eggs thus treated from developing to a larval stage. Solutions weaker than 0.7 vol. per cent. permit practically all eggs to proceed to

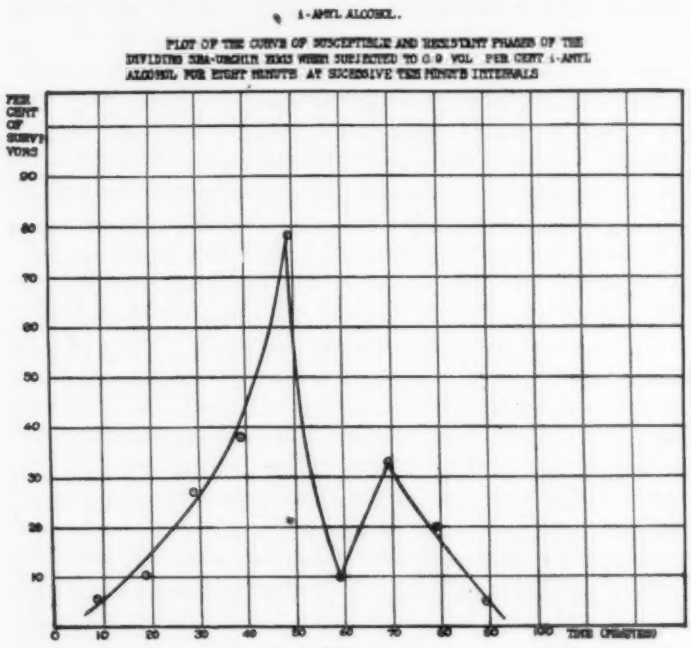

Fig. 1.

the blastula stage with these exposures, with little appreciable difference. At 1.0 vol. per cent. not more than 20 per cent. of eggs form free-swimming blastulæ even when exposed at the period of highest resistance; (*e.g.*, 30 to 40 minutes after fertili-

zation) and above this concentration the toxicity is such that the decline in survivals is very rapid. In 1.25 vol. per cent. solutions, all eggs are killed in all stages with exposures of nine minutes.

Table I. summarizes the results of a typical series of experiments with i-amyl alcohol. This particular series of experiments was started in the afternoon of July 16, and the observations noted in the third column were carried over into the morning of

TABLE I.

I-AMYL ALCOHOL.

July 16, 1:45 P.M. The fertilized eggs were placed at the intervals after fertilization noted in column 1 in 50 cc. of 0.9 vol. per cent i-amyl alcohol. Al exposures except the first (1) (6 minutes) were of eight minutes duration.

Intervals After Fertilization.	Observed Condition of the Eggs at the Time of Removal from Sol.	Proportion Forming Blastulæ and Condition of Remaining Eggs Next Day.
(1) 3– 9 m.	Fertilization-membranes well formed. No marked cytolytic change noted.	About 5 per cent. form free swimming blastulæ. Considerable numbers cytolyzed.
(2) 10–18 m.	No marked change in appearance. Uniform.	About 10 per cent. form blastulæ. Not so badly cytolyzed; most cells intact.
(3) 20–28 m.	Membranes markedly swollen in some cases. Slight fading of pigment.	Nearly 30 per cent. free-swimming blastulæ. Most eggs intact.
(4) 30–38 m.	A few cells plasmolyzed, other membranes markedly swollen.	Between 30 and 40 per cent. free-swimming blastulæ. Most others intact but swollen.
(5) 40–48 m.	No marked change. Faint indication of cleavage furrow in a few scattered cells.	Large majority (75–80 per cent.) form swimming blastulæ.
(6) 50–58 m.	About 65 per cent. have entered the two-celled stage. Some show shrinkage.	Relatively few (less than 10 per cent.) form surviving blastulæ.
(7) 60–68 m.	About 90 per cent. in two-celled stage. Others intact.	Between 30–35 per cent. form blastulæ.
(8) 70–78 m.	Practically all in two-celled stage.	Few (15–20 per cent.) form blastulæ. Others intact.
(9) 80–88 m.	A few are starting second cleavage furrow.	3–5 per cent. form blastulæ. Others intact.

the following day. A similar series of experiments performed at about the same time with the same alcohol in somewhat lower concentration (0.8 vol. per cent.), but with slightly longer (10-minute) exposures yielded substantially the same results. On the following day experiments were carried out on eggs subjected to 1.1 vol. per cent. solutions with only brief (3-, 4- and 5-minute) exposures with the results noted above.

In the controls about one half of the eggs were in the two-celled stage at fifty-three minutes after fertilization, and at sixty-five minutes between 85 and 90 per cent. were divided. There is a definite period of well-marked susceptibility immediately following fertilization; the susceptibility then gradually and progressively declines up to the end of forty-eight minutes (just before the first cleavage). There then follows a very susceptible period just at the time of cleavage. Later the resistant phase reappears until about the time of second cleavage. If the time intervals are plotted as abscissæ, and the percentage of surviving blastulæ as ordinates, the relationships may be represented in the curve shown in Fig. 1.

Hexyl Alcohol.

In exploring the range of suitable concentrations for hexyl alcohol, the next higher member of the series, assuming that it should be approximately three times as effective as i-amyl alcohol, three preliminary experiments were performed. For these, solutions of 0.1, 0.25 and 0.30 vol. per cent. were used respectively. The time of exposure was shortened to five minutes, for the reason that it was thought the concentrations were, if anything, a little above the optimum. The results clearly showed that the solutions of 0.1 vol. per cent. was not sufficiently toxic to demonstrate any variation of susceptibility in the eggs, since at whatever period they were exposed practically all eggs survived to the free-swimming blastula stage. On the other hand, the two higher concentrations proved too toxic, so that practically none of the eggs continued their development after subjection to these solutions at any period. The 0.25 vol. per cent. solution, although it suppressed further development, was not quite intense enough in its action to cause cytolysis in the eggs, with few exceptions. The 0.30 vol. per cent. concentration caused very evident cytolysis, and rupture was almost universal. Accordingly, series of experiments were carried out to test the various concentrations between 0.1 vol. per cent. and 0.25 vol. per cent. Two of these experiments are summarized in Table II., and may be regarded as typical.

These results show a much less definite evidence of a rhythm of

TABLE II.

HEXYL ALCOHOL.

August 11, 10:15 A.M. Fertilized Arbacia eggs were placed at intervals noted in 50 cc. of 0.13 and 0.17 vol. per cent. of Hexyl alcohol respectively, and allowed to remain in them for five minutes. They were then placed in watch glasses, quickly observed, and treated as described in the previous experiment.

Intervals After Fertilization.	(A) 0.13 Vol. Per Cent.		(B) 0.17 Vol. Per Cent.	
	Observed Condition.	Proportion Forming Blastulæ.	Observed Condition.	Proportion Forming Blastulæ.
(1) 15–20m.	Fert. membrane well formed. No marked cytolysis noted.	Majority (90 per cent.) form blastulæ.	No marked cytolysis. Uniform batch of fertile eggs.	Between 70 and 80 per cent. form blastulæ. Other cytolyzed but intact.
(2) 25–30m.	No marked change.	Between 85–90 per cent. form swimming blastulæ	Fert. membrane in most eggs swollen; no great change otherwise.	70–75 per cent. blastulæ. Few ruptured.
(3) 35–40m.	Membrane swollen, few show slight plasmolysis.	Practically all (90 per cent. or over) form blastulæ. Others intact.	No marked change noted.	90 per cent. form blastulæ.
(4) 45–50m.	Slight loss in pigmentation. No marked cytolysis however.	90 per cent. form blastulæ. Few ruptured.	Slight fading of pigment. No other marked change.	Between 65–70 per cent. form blastulæ. Few scattered cells ruptured.
(5) 55–60m.	About half in two-celled stage. No marked change.	80–85 per cent. form blastulæ. Others mostly intact.	All intact, 50 per cent. or over in two-celled stage.	Nearly 60 per cent. form blastulæ.
(6) 65–70m.	Fully 85 per cent. in two-celled stage; no marked change from preceding.	About 76 per cent. form swimming blastulæ.	No change.	Not more than 50 per cent. form blastulæ. Others badly cytolyzed but mostly intact.
(7) 75–80m.	No marked change.	Between 65–70 per cent. swimming blastulæ; others badly cytolyzed.	Slight loss of pigment. No marked change.	About 60 per cent. blastulæ. Two-celled eggs conspicuous. Most others intact.
(8) 85–90m.	Few cells (ca. 5–7 per cent.) show second cleavage. No marked change.	About 60 per cent. form swimming blastulæ. Most others intact.	Few cells in second cleavage. No marked cytolysis.	Nearly 85 per cent. swimming blastulæ.

susceptibility than those just described for i-amyl alcohol. The eggs apparently maintain throughout the cycle a relatively high resistance to the concentrations of hexyl alcohol here used, with only a slight increase of susceptibility at the time of

first cleavage; in the one case (0.17 vol. per cent.) there is evidence of a slight return of resistance just afterwards, and in the other (0.13 vol. per cent.) there is not. Why there should be this difference in the behavior of the two alcohols is difficult to explain. The exposures to hexyl alcohol were perhaps insufficiently

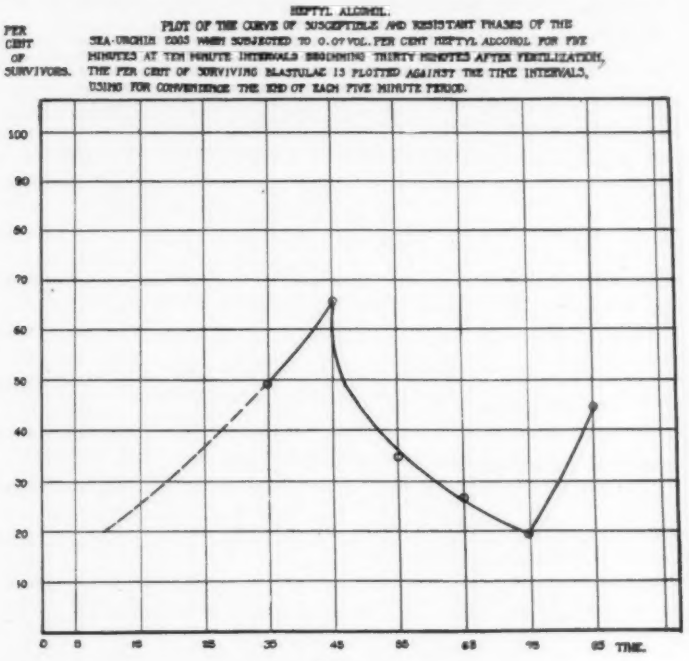

FIG. 2.

prolonged to bring out a well marked differential effect at the different stages of the cycle. Again, it is well known that certain anæsthetics are less effective than others in suppressing the cell-division process; also many neuromuscular responses react differently to a given anæsthetic in different animals, and in the same animal at different ages. Thus Lillie[1] found chloretone much less effective than chloral hydrate in suppressing cleavage in Arbacia eggs. As regards the alcohols that he tried, he lists propyl, butyl, amyl in the order of increasing favorability, while

[1] R. S. Lillie, *Jour. Biol. Chem.*, 1914, Vol. 17, No. 2, p. 130.

ethyl and capryl show a higher toxicity than the others.[1] It may be that differences,—both qualitative and quantitative—in the lipoid elements of the tissues, and hence in the plasmamembrane, form the basis of the observed physiological difference.

In the controls which were running parallel to the two experiments just described, a majority of the eggs entered the two-celled stage at about fifty-eight minutes after fertilization. At sixty-five minutes after fertilization, between 85 and 90 per cent. had cleaved. Practically all eggs in the controls had reached the blastula stage the following day.

Heptyl Alcohol.

A series of six experiments were performed with this alcohol to determine the limits of suitable concentration. Reasoning from the data in the proceeding, it was thought that the optimum concentration would be in the proximity of between 0.04 and 0.07 vol. per cent., but to make sure, concentrations as low as 0.02 and as high as 0.08 vol. per cent. were used. As a matter of fact, solutions of 0.06 and 0.07 vol. per cent. were found to be the best suited to the experiments, although it is interesting to note that even weaker solutions showed marked toxic action on eggs at the time of formation of the first cleavage furrow, while during the stages preceding and succeeding division, they had relatively little influence. Table III. summarizes three experiments with this alcohol, and is fairly typical of results obtained in other experiments. By some unavoidable oversight in technical procedure, records for the first two periods (*i.e.*, respectively 10 and 20 minutes after fertilization) were not obtained, but from the results of other series it is evident that the eggs maintain a rather high resistance at these times. The results given in Table III. show that 0.07 vol. per cent. approximates the favorable concentration for this alcohol, while the solutions on either side are slightly hypo- and hyper-toxic respectively; *i.e.*, in the one case practically all eggs survive to the blastula stage, and in the other nearly all die. Recovery of resistance after the first division appears to be relatively slow. When the percentage of surviving blastulæ is plotted against the time intervals, regarding

[1] See *ibid.*, p. 133.

TABLE III.

HEPTYL ALCOHOL.

Fertilized *Arbacia* eggs were placed in 50 cc.. of the following solutions of heptyl alcohol at ten minute intervals, and allowed to remain in each solution (0.06, 0.07 and 0.08 vol. per cent.) for five minutes. They were then treated as previously described.

Intervals.	(A) 0.06.		(B) 0.07.		(C) 0.08.	
	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.
(1) 30–35 m.	No marked cytolysis. Membranes slightly swollen.	70–75 per cent. free-swimming blastulæ. Few ruptured.	Membranes slightly swollen. No appreciable loss of pigment.	About 48–50 per cent. swimming blastulæ. Other cells ruptured in many cases.	Noticeable loss of pigment. Membranes markedly swollen, cytoplasm shows slight cretination.	Not more than 1 per cent. swimming blastulæ Majority intact.
(2) 40–45 m.	No marked change.	About 82 per cent. swimming blastulæ. Others intact.	Slight loss pigment. No other marked change.	Nearly 65 per cent. swimming blastulæ. Few ruptured.	About as noted above.	Practically no swimming blastulæ. Most badly cytolyzed.
(3) 50–55 m.	Between 25–30 per cent. show furrow. Some show loss of pigment.	88–90 per cent. active. Some ruptured but still active.	No marked change.	Relatively few (33–35) per cent. active. Whole masses of cells ruptured. None of two-celled stage stage seen in field. seen in field.	Marked loss of pigment. All intact however.	Less than 1 per cent. active. Others badly cytolyzed but mostly intact.
(4) 60–65 m.	About 80 per cent. in or entering two-celled stage. No great changes noted.	About 75 per cent. active. Considerable number remain in two-celled stage but are intact.	Nearly 80 per cent. in cleavage. Rather noticeable loss of pigment. All intact.	Between 24–28 per cent. active blastulæ. Marked cytolysis, with all stages of rupturing. Rather striking condition.	Marked loss of pigment. Majority cleaving.	Only a few survivors. Others ruptured, and none of the two-celled eggs intact.

TAPLE III.—*Continued.*

Intervals.	(A) 0.06.		(B) 0.07.		(C) 0.08.	
	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.
(5) 70–75 m.	Majority have cleaved, no marked cytolysis noted.	Between 85–90 per cent. active. A few cells are ruptured. Others rather badly cytolyzed.	90 per cent. two-celled. Slight loss of pigment noted.	Not over 20 per cent. active. Most others badly cytolyzed, a few ruptured.	Majority as before in two-celled stage. Pigment faded.	All badly cytolyzed, over half ruptured.
(6) 80–85 m.	Considerable number show second furrow.	Few active blastulæ. Large number ruptured but most two-celled eggs intact.	No marked change.	Between 40–50 per cent. active glastulæ. Others intact although badly cytolyzed.	Pigment loss characteristic. No other marked cytolytic change.	About 5 per cent. active blastulæ. Others ruptured.

as typical the data of the 0.07 vol. per cent. solution, an interesting curve is obtained (Fig. 2) which is fairly comparable with the one shown for i-amyl alcohol. There is a gradual rise in resistance up to the period of first cleavage, with a sharp drop during cell-division followed by a slow recoverry.

Octyl Alcohol.

Normal octyl alcohol is apparently considerably more toxic than its isomere capryl alcohol. In a series of five experiments with octyl alcohol in concentrations ranging from 0.010 to 0.030 vol. per cent., the best concentration for five minute times of exposure was found to be in the neighborhood of 0.015 vol. per cent. On the other hand the outcome of fourteen experiments with capryl alcohol showed the optimum concentration for the same time of exposure to be between 0.035 and 0.045 vol. per cent., which is between two and three times the favorable concentration of normal octyl alcohol. Table IV. summaries a

Table IV.

Normal Octyl Alcohol.

Fertilized eggs were subjected for five minutes to 0.013 vol. per cent. of normal octyl alcohol at intervals of ten minutes.

Intervals After Fertilization.	Observed Condition on Removal from Fluid.	Observed Condition the Following Day.
(1) 15–20 m.	Fertilization membrane well formed. Slight loss of pigment.	Nearly 50 per cent. active blastulæ. Others badly cytolyzed, few ruptured.
(2) 25–30 m.	No noticeable cytolysis although very marked loss of pigment.	About 65 per cent. active blastulæ. Others cytolyzed.
(3) 35–40 m.	Decided loss of pigment. No marked change in membrane or cytoplasm.	Nearly 80 per cent. active. Others intact.
(4) 45–50 m.	About 2 per cent. show first furrow. Slight loss pigment. No cytolysis.	Practically all active blastulæ.
(5) 55–60 m.	Over half in first cleavage.	About 85 per cent. active.
(6) 65–70 m.	About 90 per cent. in two-celled stage.	Almost 60 per cent. active blastulæ, numbers of two-celled egg present and mostly intact. Some badly cytolyzed and ruptured.
(7) 75–80 m.	Aside from loss of pigment no noticeable change.	Between 65 and 70 per cent. active blastulæ. Most others cytolyzed but intact.
(8) 85–90 m.	A few (1 per cent.) just begin to show second cleavage furrow. No marked change in appearance.	Between 85 and 90 per cent. active blastulæ. Others cytolyzed but intact.

typical experiment using normal octyl alcohol of 0.013 vol. per cent. concentration. For exposures of five minutes duration, this concentration gave the best results, and showed very clearly the resistant and susceptible phases.

Capryl Alcohol.

As mentioned before, experiments with various concentrations of capryl alcohol showed that for brief exposures, the most favorable concentration was nearly three times that of normal octyl alcohol. This may perhaps be accounted for in some measure by the fact that not all samples of capryl alcohol are

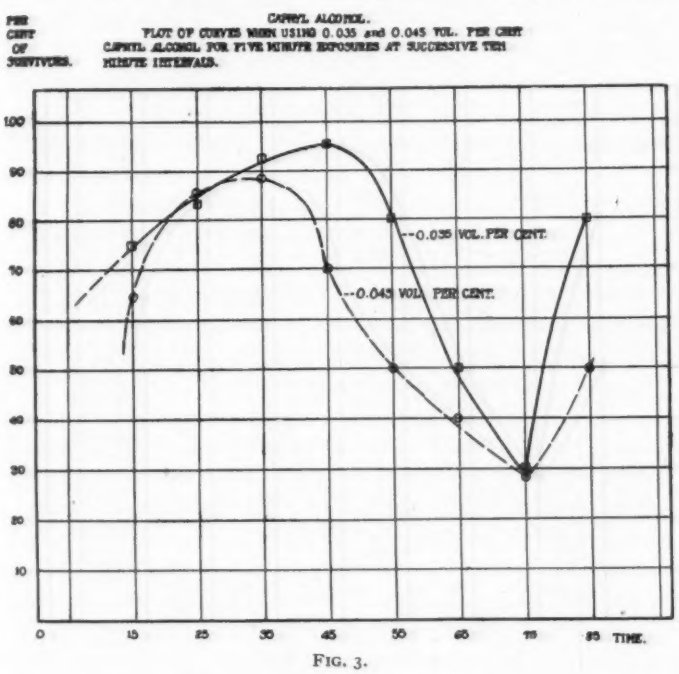

FIG. 3.

uniform in chemical composition and purity; a slight difference in this respect is known to make a decided difference in its chemical and physiological activity. In suitable concentrations, this alcohol is without doubt one of the most satisfactory for

showing susceptible and resistant phases in dividing eggs. Several experiments were tried with this alcohol in which the concentration was kept constant and the time of exposure was varied; and from the data thus gathered, it seems probable that of the two factors, concentration is the more important. In other words, if the concentration is such that it gives the best

TABLE V.

Fertilized eggs were subjected for five minutes at ten minute intervals to 0.035 and 0.045 vol. per cent. capryl alcohol.

Intervals.	(A) 0.035 Vol. Per Cent.		(B) 0.045 Vol. Per Cent.	
	Observed Condition.	Condition Following Day.	Observed Condition.	Condition Following Day.
(1) 10–15m.	Membranes well formed. No great difference from normal eggs.	75–80 per cent. active. Others cytolyzed but mostly intact.	No marked difference from normal eggs.	About 65 per cent active. Others badly cytolyzed.
(2) 20–25m.	Membrane slightly swollen. No marked cytolysis.	About 83 per cent. active. Others badly cytolyzed.	No marked change.	Nearly 85 per cent. active. Others intact.
(3) 30–35m.	Few show loss of pigment, cytoplasm shrunken in some cases.	About 92 per cent. active. Others intact.	Marked fading of pigment. No marked cytolysis.	Between 85–90 per cent. active. Some ruptured eggs motile.
(4) 40–45m.	No marked change. None show cleavage furrow.	About 95 per cent. active blastulæ. Others intact.	No marked change, slight loss of pigment.	About 70 per cent active. Others mostly intact.
(5) 50–55m.	Nearly half show first cleavage furrow No marked change.	80 per cent. active blastulæ. Few ruptured.	No marked change.	About 50 per cent. active blastulæ. Most others badly cytolyzed, many ruptured.
(6) 60–65m.	Nearly all show first cleavage furrow. No marked cytolysis.	About 50 per cent. active blastulæ. Most others ruptured.	Practically all in two cell stage. Some loss pigment.	About 40 per cent. active. All others badly ruptured. Some still in two cells.
(7) 70–75m.	No marked change. Few scattered cells show second furrow.	About 30 per cent. active blastulæ. Others badly cytolyzed.	No marked change, except loss of pigment.	Nearly 30 per cent. active. Others badly cytolyzed. Few persist in two cells.
(8) 80–85m.	No marked cytolysis.	Nearly 80 per cent. active blastulæ. Others mostly intact.	No marked change.	Nearly 50 per cent. active blastulæ. Others cytolyzed but mostly intact.

When plotted the data gives interesting curves as shown in Fig. 3.

results with a five-minute exposure, when the exposure is prolonged to eight minutes, very little or no difference is detected. This generalization, however, could probably be applied only within narrow limits.

The data from two experiments using capryl alcohol in 0.035 and 0.045 vol. per cent. concentrations respectively are given in Table V. These records are fairly typical of results of other experiments.

Summary.

1. The developing sea-urchin egg when subjected to suitable concentrations of various lipoid-soluble substances—i-amyl, hexyl, heptyl, octyl and capryl alcohols—shows unmistakable rhythms of susceptible and resistant phases, which when taken in connection with the earlier observations of Lyon, Herlant, Mathews, Spaulding, Lillie and others, constitute additional evidence that a very intimate relation exists between the general physiological condition of the egg, and the physical state of its plasma-membrane.

2. During the first ten or fifteen minutes after fertilization the eggs are more susceptible than at any other time until the period just preceding division. A comparatively resistant phase gradually becomes more and more marked up to just before the first cell-division (about 45 or 48 minutes after fertilization). This is followed by a period of decidedly increased susceptibility which lasts for about 15 or 20 minutes, during which time marked cytological effects are noted. Subsequently the resistant phase is largely recovered, and maintained up to the time of the second cleavage.

3. The most favorable concentrations of the various alcohols for demonstrating the rhythm of susceptibility range as follows: i-amyl, between 0.7 and 0.9 vol. per cent.; hexyl, between 0.13 and 0.17 vol. per cent.; heptyl, between 0.06 and 0.07 vol. per cent.; normal octyl, about 0.015; while capryl was considerably above its isomere (normal octyl) between 0.035 and 0.045 vol. per cent. The best records were obtained in experiments using i-amyl and capryl alcohols, possibly indicating a higher specific toxicity of these when compared to the others.

4. When suitable concentrations were used, no marked

differences could be detected by varying slightly the durations of exposure. Eggs exposed for five, eight or even ten minutes to the same concentration gave similar results. This, however, would probably apply only within narrow limits.

LIGHT PRODUCTION IN CEPHALOPODS, I.

An Introductory Survey.

S. STILLMAN BERRY,

Redlands, California.

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1. Introduction.

Recent interest in the subject of biophotogenesis has been so great, and bids so fair to continue at high ebb, until at least the problem of the economical artificial production of chemical light has been solved, that for the use of the many classes of investigators, most of whom are not zoölogists and can scarcely be expected to possess accurate taxonomic knowledge of the group with which they may chance to wish to work, it would be exceedingly desirable if there could be placed on record in compact form a summary of all the species of each principal division of plants and animals which are known or thought to possess photogenic properties. The writer's desire to see this service performed on behalf of the cephalopods, animals which must always stand well up with the highest in the estimation of the student of organic light, furnished the initial stimulus which has finally broadened into the production of the present paper. As a taxonomist, however, and, in so far as this particular group of

animals is concerned, one of that despised species, the "closet naturalist"—he can only go a certain way with his subject, and by the same token, his remarks must perforce have only a very limited value. Yet the effort seems worth while spending, and he can fairly plead in extenuation of his temerity, if not of his own limitations, that cephalopods are such active, delicately balanced creatures, and so exquisitely adjusted to an environment in which it is next to impossible to observe them accurately, and which it is even more vain to attempt to establish, even partially, under artificial conditions, that the difficulties of subjecting the details of their life history and ecology to that searching examination required by the standards of modern biological investigation have proven practically insurmountable. Therefore the unfortunate circumstance that we have no specialist in this branch,—no authoritative student of the bionomics of cephalopods, and that such halting summarization as can be done must be handled by the systematist or general student of the group, if at all. Admitting then the largely pragmatic and temporary rather than permanently intrinsic value of the present dissertation we may proceed with it, for even so small a contribution as this can pretend to be should prove helpful.

Amid the wealth of remarkable features, structural and physiological, with which the entire group of the Cephalopoda entices the student, the variety and multiplication of those which in an earlier day would have been as unquestioningly as delightedly hailed as *adaptive* are supremely conspicuous. These are special for the most part to the conditions and vicissitudes brought about by an exceptionally active manner of life in an environment full of actual or potential diversity. Not even the fishes are better swimmers, nor, with all their aristocratic vertebrate organization, lead a more complicated struggle for existence. It is perhaps concomitantly both a result and cause of all this that the group so fairly teems with bizarre cells, tissues, organs, complexes of organs, which, as we may as well admit without further parley, can scarcely be interpreted otherwise than as marvelously exquisite adaptations, each to its own definite end. Such knowledge of most of these as we possess has been amassed almost wholly since the time of Darwin, else the pages of the

"Origin" might have been enriched by many examples as startling in their way as any of the classical ones. The complex cephalopod chromatophore, the inter-playing system of exactly balanced musculature with scarcely any hard skeletal parts to give it support and leverage which goes to make up the arms and each single sucker, the delicate adjustment between eye, sucker and chromatophore through the mediation of the nervous system to result in one of our most perfect demonstrations of concealing coloration, the innumerable types of hectocotylus often involving the most astonishing modifications in sexual behavior, the amazing and still insufficiently understood mechanism of the spermatophore, the eyes, and, without attempting to prolong the list further, the photogenic organs,—each is in its own way a triumph of adaptive development, how much so we may perhaps infer to some extent from the widespread occurrence of these structures in one form or another among nearly all the now-surviving cephalopods. Continuing with the structures last named, for instance, I think it can be truly said that no other class of animals can compare with the cephalopods in the complexity, diversity, beauty, brilliancy,—in brief, the high specialization of organs devoted to the production and utilization of that form of energy which to our human faculties finds expression as light.

It has been said with considerable show of truth that the generation of light by the plasm of animals and plants is really far less to be marveled at than the transformation of their energy into motion. But motion is practically a general property of protoplasm in all its forms, without which it could scarcely exist as living substance at all. The reason why the production of organic light appears so remarkable to most of us is more special: it is partly of course because of the apparent economy of this light so far as the dissipation of heat energy is concerned, but mainly to the average observer because of its evident highly specialized adaptation to certain particular ends.

Dubois wrote in 1895:[1] "The most resplendent of all animals are *insects*, of which class the glowworm, beloved of the poets, is one of the most brilliant examples." Cephalopods were scarcely

[1] Smithsonian Report, 1895, p. 418.

noticed as being luminous at that period, but now we know that the firefly and glow-worm pale in comparison, and that probably not even the brilliant display of the tropical elaters can vie with the gorgeous pyrotechnics of certain squids. It is indeed quite possible that the latter exhibit the highest development of the photogenic function known in the entire animal kingdom.

2. Classification of Cephalopods.

Living members of the Molluscan Class Cephalopoda cleave simply and naturally into two well-defined, easily separable groups. The first of these, and that universally regarded as the most primitive, is the Order Tetrabranchiata, comprising only the few species contained in the single genus *Nautilus*. The animals of this group are characterized especially by the possession of a massive, chambered, external shell; of a "funnel" formed by the appression of two lateral folds which remain unfused in the median line below; of a system of suckerless lobes around the mouth, bearing retractile, annulated tentacles; of such traces of metamerism as the presence of two pairs each of ctenidia, "branchial hearts," auricles, renal organs, and osphradia; and of a simple "pin-hole" eye, open to the exterior. Ink-sac and chromatophores are absent.

The second group, Order Dibranchiata, comprising all living cephalopods except *Nautilus*, is characterized by either the complete atrophy of the shell or its reduction in the adult to a concealed loose coil (Spirulidæ), a calcareous plate (Sepiidæ), or a horny pen; by an entire, tubular funnel; by the development of the anterior portion of the primitive foot into a series of eight or ten muscular, sucker-bearing, tentacle-like arms about the mouth; by but a single pair each of ctenidia, branchial hearts, auricles and renal organs; by the presence, at least typically, of highly developed eyes; by the development of complex, specialized pigment cells in the skin; and by the presence of the peculiar "ink-sac." Osphradia are absent.

The Dibranchiata in their turn are sharply divisible into two subgroups, the Decapoda and Octopoda. The former are mainly pelagic; have finned, generally elongate bodies; have not only the eight "primary" arms of the octopods, but a pair of

specially modified "tentacles" as well; have the suckers pedunculate, reinforced with a chitinous ring, and often very curiously modified; and have a so-called buccal membrane surrounding the mouth.

The Octopoda are principally shore or bottom-loving forms; short bodied; lack fins, or have them only secondarily developed; have eight arms only, with their suckers sessile and lacking chitinous rings; and lack the buccal membrane. There are also important internal characters which need not concern us here, but it may be said that few living groups are more sharply delimited. As a whole the group of the Decapoda has seemed to most students more archaic than the Octopoda, at any rate is less uniformly divergent from what must have been the ancestral stock, but it includes many highly specialized types, and probably neither group as we now know it can be taken as especially "primitive."

Of the two groups the Decapoda are much the less uniform, and are therefore still further to be divided. The classical bifurcation, which has found general acceptance until quite recently, is that of d'Orbigny, into Œgopsida, or those forms in which there is a free eyelid, and the Myopsida, in which there is a simple fold-like pseudo-lid, or the skin passes uninterruptedly over the eyeball. There seems to be no doubt but that the Œgopsida, at least, form a monophyletic, natural group, for on this point there is reasonable agreement. This cannot be said of the Myopsida.

The principal alternative system is that of Naef (1916).[1] He sets apart the Sepias and Sepiolids together as one of the two main subgroups, the Sepioidea. The other group, which not altogether satisfactorily he denominates Teuthoidea, is subdivided into Myopsida (here restricted by the elimination of the Sepioidea and hence comprising only the single family Loliginidæ) and Œgopsida, the latter as outlined above. Naef's classification avoids several very serious difficulties involved in the standard arrangement, but encounters, perhaps, certain others, which need not be dwelt upon here.

For convenience the two systems are summarized in the

[1] Naef, A. J., *Pubblicazione Stazione Zoologica Napoli*, V. 1, pp. 14–17, 1916.

accompanying table, that of Naef being slightly modified from his printed synopsis in order better to serve the purposes of the moment.

STANDARD SYNOPSIS OF THE CLASS CEPHALOPODA.

Phylum MOLLUSCA.
Class CEPHALOPODA.
Order I. *Tetrabranchiata.*
Suborder 1. *Nautiloidea.*
Order II. *Dibranchiata.*
Suborder 1. *Decapoda.*
Division A. *Œgopsida.*
Division B. *Myopsida.*
Suborder 2. *Octopoda.*
Division A. *Pteroti.*
Division B. *Apteri.*

SYNOPSIS OF THE CLASS CEPHALOPODA ACCORDING TO NAEF.

Phylum MOLLUSCA.
Class CEPHALOPODA.
Sub-class I. *Tetrabranchiata.*
Order 1. *Nautiloidea.*
Sub-class II. *Dibranchiata.*
Order 1. *Decapoda.*
Suborder A. *Teuthoidea.*
(*a*) *Teuthoidea myopsida.*
(*b*) *Teuthoidea ægopsida.*
Suborder B. *Sepioidea.*
Order 2. *Octopoda.*
Suborder A. *Pteroti.*
Suborder B. *Apteri.*

In number of families and genera now living, the Œgopsida easily preponderate, but the tremendous modern development of the genera *Polypus*, *Sepia* and *Loligo* throws the preponderance in species over to the side of the Myopsids (+ Sepioids) and Octopods. For instance, among recent Cephalopoda are to be recognized some 32 families, and in round figures about 120 genera and 600 species.[1] Of these the Œgopsid Decapods claim 16 families (one half the total), 66 genera (slightly over one half), and around 175 species (nearly one third). The Myopsid (+ Sepioid) Decapods account for but 7 families (nearly one quarter), and 27 genera (nearly one quarter), but

[1] More critical figures compiled from the author's card register will be found in Tables I., II. and III. to follow.

include around 225 species (over one third of the total). The Octopoda, with only 8 families (one quarter) and 25 genera (slightly over one fifth), yet develop nearly 200 species (one third of the total). In comparison to this the single family and genus of Tetrabranchiata (3 generally recognized species) hold a minor place in the fauna.

The extraordinary development of the Œgopsida in families and genera is indicative, as the reader may anticipate, of the more than usual modification to which the different branches of this group have been subject, and we are to see that this is particularly true of the photogenic organs. The number of species on the other hand seems to have been held down by the circumstance that a very considerable proportion of the genera are pelagic types of widespread distribution, and, like so many other animals showing somewhat similar ecologic relations, do not break up well into species with our present degree of refinement in perception.

This brief survey of the classification may seem a digression, but without it as a guide, no consideration of the distribution of the photogenic function within the group as a whole or within its components could be entirely intelligible.

3. Distribution of the Photogenic Function among Cephalopods.

By no means all cephalopods are luminous. Among the entire major division of octopods but two species, *Melanoteuthis lucens* Joubin and *Eledonella alberti* Joubin, have been described as possessing photogenic organs, while even here the fact that the structures so described are actually designed for the production of light still remains to be demonstrated. The various instances where octopi have been observed to emit light are almost always poorly authenticated, though it is not impossible that in some cases, such as the observation by Darwin during the Voyage of the "Beagle," which will be noted later, are explicable on the assumption of infection by photogenic bacteria or protozoa. However that may be, and perhaps the point is not yet definitely settled, in the morphological evidence offered the two species mentioned stand quite alone.

On the other hand among the decapods photogenic properties are so widespread that taking the class as a whole, and even including the Octopoda, I am aware of no other major division of metazoan animals which shows such a proportional development of luminous species as the Cephalopoda.

It is therefore no wonder that the scattered literature, as well as the fragmentary character of the information to be gleaned therefrom, offers almost insurmountable difficulties to the inquiring student, while even reasonably complete information is scarcely to be found in any text-book or work of reference. There seems in fact no more recent effort on the part of teuthologists to meet this need than that of Chun (:10), which seems to have been carried out simply as an incident to the preparation of his monumental work on the "Valdivia" Œgopsids, an expensive and in the United States a relatively inaccessible volume. Furthermore he dealt with but one group of cephalopods, while even for this group there has now accumulated a considerable mass of additional information. In the English language by all odds the best and most trustworthy summary is the brief one of Hoyle (:08).

Probably the best way to convey an accurate idea of the manner of distribution of the photogenic function within the group is to present summarily a systematic survey of the entire class, carried down at least as far as genera, and including at the same time such appropriate supplementary data concerning the possession of this function as in each case is possible. An effort to do this is constituted in the following synopsis. In all cases an attempt is made to state as exactly as one writer can the number of valid or recognized species in each genus, and, in the case of photogenic forms, a full list of the species themselves, as well as an indication of the situation of their photophores. In a catalogue of this kind it must at the outset be admitted that the figures given are only approximate, due to the fact that entire elimination of the personal element is quite impossible, even though the concrete numbers quoted are not mere estimates, but represent in every instance an actual weighing of the validity of each specific name in the light of all available information, information which from the very nature of this paper cannot be

gone into in any detail here. The attempt has been made to be both conservative and judicial, but it of course goes without saying that the result attained must be regarded as far from final. Aside from matters of judgment even, it would be presumptious to claim for this list either completeness or freedom from error, but every effort has been made to reduce unnecessary mistakes to a minimum, and after all it is utility rather than finality which must always be remembered as the end in view. In certain special cases where the number of valid species seems to be more than usually problematic, this circumstance has been so indicated by the use of + or ± signs.

TABLE I.[1]

SYNOPTIC TABLE OF THE CLASS CEPHALOPODA, SHOWING THE OCCURRENCE OF PHOTOGENETIC ORGANS.

Class **CEPHALOPODA.**

Order *TETRABRANCHIATA.*

Suborder NAUTILOIDEA.

Family *Nautilidæ.*

Genus *Nautilus* Linnæus 1758. (No photogenic species known.)

3 or 4 species.

Order *DIBRANCHIATA.*

Suborder DECAPODA.

Division ŒGOPSIDA.

Superfamily Architeuthoidea.

Family *Architeuthidæ.* (No photogenic species known.)

Genus *Architeuthus* Steenstrup, 1857.

14 species.

Superfamily Enoploteuthoidea.

Family *Gonatidæ.* (No photogenic species known.)

Genus *Gonatus* Gray, 1849.

3 species.

Family ONYCHOTEUTHIDÆ.

Genus *Onykia* Lesueur, 1821. (No photogenic species known.)

12 ± species.

Genus ONYCHOTEUTHIS Lichtenstein, 1818. (2 axial photogenic organs in pallial chamber.)

1 established species, *banksii;* several doubtful.

Genus *Tetronychoteuthis* Pfeffer, 1900. (No photogenic species known.)

2 species.

Genus CHAUNOTEUTHIS Appellöf, 1891. (Photogenic organs in ventral integument of mantle—*Chun;* no luminous organs—Pfeffer.)

1 species: *mollis.*

[1] Photogenic families and genera are printed in small capitals.

Genus *Ancistroteuthis* Gray, 1849. (No photogenic species known.)
1 species.
Genus *Moroteuthis* Verrill, 1881. (No photogenic species known.)
3 species.

Family LYCOTEUTHIDÆ.
Genus LYCOTEUTHIS Pfeffer, 1900. (Photogenic organs on eyes, in stalks of tentacles, and in pallial chamber.)
2 species: *diadema, jattai;* possibly identical.
Genus NEMATOLAMPAS Berry, 1913. (Photogenic organs on eyes, in arms, in stalks of tentacles, in pallial chamber, and at posterior tip of body.)
1 species: *regalis.*

Family LAMPADIOTEUTHIDÆ.
Genus LAMPADIOTEUTHIS Berry, 1916. (Photogenic organs on eyes, in stalks of tentacles, and in pallial chamber.)
1 species: *megaleia.*

Family ENOPLOTEUTHIDÆ.
Genus ENOPLOTEUTHIS d'Orbigny, 1844. (Photogenic organs on eyes and in the integument of mantle, funnel, head, and arms, but almost entirely confined to ventral aspect.)
3 species: *leptura, chunii, galaxias.*
Genus ABRALIA Gray, 1849. (Photogenic organs on eyes and in the integument of arms, head, funnel and mantle, but almost entirely confined to ventral aspect.)
7 species: *andamanica, armata, astrolineata, astrosticta, steindachneri, trigonura, veranyi.*
Genus ABRALIOPSIS Joubin, 1896. (Photogenic organs on eyes, at tips of ventral arms, and in the integument of arms, head, funnel, and mantle, but almost entirely confined to ventral aspect.)
5 + species: *affinis, hoylei, lineata, morisii, owenii, pfefferi.*
Genus WATASENIA Ishikawa, 1914. (Photogenic organs as in *Abraliopsis.*)
1 species: *scintillans.*
Genus ENOPLOION Pfeffer, 1912. (Larval form; photogenic organs on tentacle stalks and ventral integument of ventral arms, head, funnel, and mantle.)
1 species: *eustictum.*
Genus ASTHENOTEUTHION Pfeffer, 1912. (Larval form; photogenic organs on eyes.)
1 species: *planctonicum.*
Genus ANCISTROCHEIRUS Gray, 1849. (Photogenic organs in ventral integument of mantle.)
1 species: *lesueurii.*
Genus THELIDIOTEUTHIS Pfeffer, 1900. (Photogenic organs on tentacle stalks and ventral integument of head and mantle.)
1 recognized species: *alessandrinii.*
Genus PTERYGIOTEUTHIS H. Fischer, 1896. (Photogenic organs on eyes, in tentacle stalks, and in pallial chamber.)
4 ± species: *gemmata, giardi, hoylei, microlampas.*
Genus PYROTEUTHIS Hoyle, 1904. (Photogenic organs on eyes, in tentacle stalks, and in pallial chamber.)
3 species: *aurantiaca, margaritifera, oceanica,* + several named larval forms.

Family OCTOPODOTEUTHIDÆ.

Genus OCTOPODOTEUTHIS Rüppell, 1844. (Photogenic organs on ink sac?)
1 species: *sicula*.

Genus *Octopodoteuthopsis* Pfeffer, 1912. (No photogenic species known.)
1 species.

Genus *Cucioteuthis* Steenstrup, 1882. (No photogenic species known.)
1 species.

Family HISTIOTEUTHIDÆ.

Genus CALLITEUTHIS Verrill, 1880. (Numerous photogenic organs in integument of arms, head, and mantle; best developed ventrally.)
12 ± species: *asteroessa, chuni, dofleini, goodrichi, hetcropsis, hoylei, japonica, meleagroteuthis, meneghini, miranda, ocellata, separata, verrilli*.

Genus HISTIOTEUTHIS d'Orbigny, 1839. (Photogenic organs as in *Calliteuthis*.)
1 species: *bonnellii*.

Genus HISTIOCHROMIUS Pfeffer, 1912. (Larval form; photogenic organs in integument of mantle on ventral aspect?)
1 species: *chuni*.

Family BENTHOTEUTHIDÆ.

Genus BENTHOTEUTHIS Verrill, 1885. (Photogenic organs on arms)
1 species: *megalops*.

Genus CTENOPTERYX Appellof, 1889. (Photogenic organs on eyes.)
1 species: *siculus*.

Superfamily Ommastrephoidea.

Family *Brachioteuthidæ*. (No photogenic species known.)

Genus *Brachioteuthis* Verrill, 1881. (+ *Tracheloteuthis* Steenstrup 1881.)
4 species.

Genus *Cirrobrachium* Hoyle, 1904(?)
1 species.

Family OMMASTREPHIDÆ.

Genus *Illex* Steenstrup, 1880. (No photogenic species known.)
2 species.

Genus *Todaropsis* Girard, 1889. (No photogenic species known.)
1 species.

Genus *Ommastrephes* d'Orbigny, 1835. (No photogenic species known.)
6 ± species.

Genus *Nototodarus* Pfeffer, 1912. (No photogenic species known.)
2 species.

Genus HYALOTEUTHIS Gray, 1849. (Photogenic organs in ventral integument of mantle.)
1 species: *pelagicus*.

Genus *Sthenoteuthis* Verrill, 1880. (No photogenic species known.)
4 species:

Genus *Symplectoteuthis* Pfeffer, 1900. (No photogenic species known.)
1 species.

Genus EUCLEOTEUTHIS Berry, 1916. (Bands of photogenic tissue on ventral aspect of head and mantle.)
1 species: *luminosa*.

Genus *Dosidicus* Steenstrup, 1857. (No photogenic species known.)
2 species.

Family *Thysanoteuthidæ*. (No photogenic species known.)
Genus *Thysanoteuthis* Troschel, 1857.
2 species.

(Position uncertain.)

Family *Lepidoteuthidæ*. (Naef refers this poorly known group to the Myopsida.)
Genus *Lepidoteuthis* Joubin, 1895. (No photogenic species known.)
1 species.

Superfamily Chiroteuthoidea.

Family *Chiroteuthidæ*.
Genus *Doratopsis* de Rochebrune, 1884. (+ *Planctoteuthis* Pfeffer, 1912, and *Leptoteuthis* Verrill, 1884; no photogenic species known.)
7 species.
Genus CHIROTEUTHIS d'Orbigny, 1839. (Photogenic organs on eyes, ventral arms, and in pallial chamber.)
7 species: ***imperator, lacertosa, macrosoma, pellucida, picteti, regnardi*** (photogenic organs undescribed), ***veranyi***.
Genus MASTIGOTEUTHIS Verrill, 1881. (Photogenic organs in integument of the arms, funnel, mantle, or fins, or even absent.)
11 species: ***dentata, famelica, levimana, magna***. (Not known to be photogenic.)
cordiformis (small tubercles, possibly photogenic, thickly distributed in dorsal integument of body).
agassizii (photogenic organs numerous in integument of head, arms, tentacle stalks and mantle, both dorsally and ventrally).
grimaldii (photogenic organs on dorsal surface of fins, and ventral surfaces of head, arms, funnel and mantle).
flammea (photogenic organs comparatively few; in integument of dorsal surface of fins, and on ventral surfaces of head, ventral arms, funnel and mantle).
talismani (photogenic organs on ventral aspect of fins).
hjorti (photogenic organs on eyes).
glaukopis (a photogenic organ in ventral border of each eyelid sinus).
Genus *Joubiniteuthis* nov.[1] (No photogenic species known.)
1 species.
Genus *Idioteuthis* Sasaki, 1916. (No photogenic species known.)
1 species.
Family *Grimalditeuthidæ*. (No photogenic species known.)
Genus *Grimalditeuthis* Joubin, 1898.
1 species.

Superfamily Cranchioidea.

Family CRANCHIIDÆ.
Subfamily Cranchiinæ. (Series of small photogenic organs on eyes.)
Genus CRANCHIA Leach, 1817.
3 species or forms: ***hispida, scabra, tenuitentaculata***.
Genus LIOCRANCHIA Pfeffer, 1884.
3 species or forms: ***globulus, reinhardtii, valdiviæ***.

[1] *Chiroteuthis Portieri* Joubin, 1916, does not seem strictly referable to the genus in which it is placed, as it is said to possess no luminous organs, while the extreme attenuation and length of the three dorsal pairs of arms are at variance with the well known state of affairs in *Chiroteuthis*. Hence I would propose the new group *Joubiniteuthis*, with this species as type.

Genus PYROGOPSIS de Rochebrune, 1884.
4 species: *pacificus, rhyncophorus, schneehageni, zygaena.*
Genus LEACHIA Lesueur, 1821.
3 species: *cyclura, ellipsoptera, eschscholtzii.*
Genus LIGURIELLA Issel, 1908.
1 species: *podophthalma.*
Subfamily Taoniinæ. (A large single or duplex photogenic organ on each eyeball.)
Genus PHASMATOPSIS de Rochebrune, 1884. (Photogenic organs not yet described.)
1 species: *cymoclypus.*
Genus TOXEUMA Chun, 1906.
1 species: *belone.*
Genus TAONIUS Steenstrup, 1861. (Photogenic organs not yet described.)
1 species: *pavo.*
Genus VERRILLITEUTHIS Berry, 1916. (Photogenic organs not yet described.)
1 species: *hyperborea.*
Genus MEGALOCRANCHIA Pfeffer, 1884.
5 species: *abyssicola, fisheri, maxima, pardus, pellucida.*
Genus LEUCOCRANCHIA Joubin, 1912.
1 species: *pfefferi.*
Genus TAONIDIUM Pfeffer, 1900. (Photogenic organs not yet described.)
4 species: *chuni, incertum, pfefferi, suhmi.*
Genus CRYSTALLOTEUTHIS Chun, 1906.
1 species: *glacialis.*
Genus PHASMATOTEUTHIS Pfeffer, 1912.
1 species: *richardi.*
Genus GALITEUTHIS Joubin, 1898.
2 species: *armata, phyllura;* possibly identical.
Genus CORYNOMMA Chun; 1906. (A pair of photogenic organs embedded in the liver in addition to the subocular photophores.)
1 species: *speculator.*
Genus HENSENIOTEUTHIS Pfeffer, 1900 (+ *Sandalops* Chun, 1906, *Helicocranchia* Massy, 1907, and *Teuthowenia* Chun, 1919.)
5 species: *antarctica, joubini, megalops, melancholicus, pfefferi.*
Genus BATHOTHAUMA Chun, 1906.
3 species: *bergeli, bouréei, lyromma.*

Division MYOPSIDA.

Superfamily Loliginoidea.

Family *Loliginidæ.* (No photogenic species known.)
Genus *Acroteuthis* Berry, 1913.
3 species.
Genus *Doryteuthis* Naef, 1912.
5 species.
Genus *Loligo* Schneider, 1784.
32 ± species.
Genus *Lolliguncula* Steenstrup, 1881.
3 or 4 species.

Genus *Loliolus* Steenstrup, 1856.
4 species.
Genus *Sepioteuthis* de Blainville, 1824.
21 ± species.

Superfamily Spiruloidea.

Family SPIRULIDÆ. (A single organ thought to be photogenic at posterior end of body.)
Genus SPIRULA Lamarck, 1799.
11 named forms (number true species uncertain): *atlantica, australis, blakei, fragilis, indopacifica, laevis, peronii, prototypus, reticulata, spirula, vulgaris.*

Superfamily Sepioidea.

Family *Promachoteuthidæ*. (No photogenic species known.)
Genus *Promachoteuthis* Hoyle, 1885.
1 species.
Family *Idiosepiidæ*. (No photogenic species known.)
Genus *Idiosepius* Steenstrup, 1881.
2 species.
Family SEPIOLIDÆ.
Subfamily Rossiinæ. (No photogenic species known with certainty.[1])
Genus ROSSIA Owen, 1834.
14 species.
Genus *Semirossia* Steenstrup, 1887.
2 species.
Subfamily Heteroteuthinæ. (A fused pair of glandular photogenic organs on ink sac in all known cases.)
Genus HETEROTEUTHIS Gray, 1849.
3 species: *dispar, hawaiiensis, weberi.*
Genus STOLOTEUTHIS Verrill, 1881. (Photogenic organs still undescribed.)
1 species: *leucoptera.*
Genus IRIDOTEUTHIS Naef, 1912.
1 species: *iris.*
Genus NECTOTEUTHIS Verrill, 1883. (Photogenic organs still undescribed.)
1 species: *pourtalesii.*
Subfamily Sepiolinæ.
Genus SEPIOLA Schneider, 1784. (Paired glandular photogenic organs on ink sac.)
11 ± species: *affinis, atlantica, aurantiaca, intermedia*(?), *ligulata, pacifica* (?), *penares* (?), *robusta, rossiæformis* (?), *sepiola, steenstrupiana.*
Genus RONDELETIA Naef, 1916. (A fused pair of glandular photogenic organs on ink sac.)
1 species: *minor.*
Genus *Sepietta* Naef, 1912. (No photogenic species.)
4 or 5 species.

[1] This lack is stated by Naef (:12, p. 245) as one of the diagnostic characters of the subfamily, but the possession of photogenic organs by *Rossia macrosoma* is definitely affirmed by Meyer (:06, pp. 390, 392). One is perforce still of unsettled mind in the matter, especially as both observers worked at Naples, and Naef goes into no details beyond the mere negation.

Genus INIOTEUTHIS Verrill, 1881. (Paired glandular photogenic organs on ink sac.)
3 species: *japonica, maculosa, parva.*
Genus *Sepiolina* Naef, 1912. (No photogenic species known.)
1 species.
Genus EUPRYMNA Steenstrup, 1887. (Paired glandular photogenic organs on ink sac.)
8 ± species: *bursa, morsei, pusilla, schneehageni, scolopes, similis, stenodactyla, tasmanica.*
Subfamily Sepiadariinæ. (No photogenic species known.)
Genus *Sepiadarium* Steenstrup, 1881.
2 species.
Genus *Sepioloidea* d'Orbigny, 1855.
1 species.
Family *Sepiidæ.* (No photogenic species known.)
Genus *Sepia* Linnæus, 1758.
80 ± species.
Genus *Metasepia* Hoyle, 1885.
2 species.
Genus *Sepiella* Gray, 1849.
11 ± species.
Genus *Hemisepius* Steenstrup, 1875.
1 species.

Suborder OCTOPODA.

Superfamily Cirroteuthoidea.

Family CIRROTEUTHIDÆ.
Genus *Cirroteuthis* Eschricht, 1836. (No photogenic species known.)
8 species.
Genus *Stauroteuthis* Verrill, 1879. (No photogenic species known.)
3 species.
Genus MELANOTEUTHIS Joubin, 1912. (A pair of supposed photogenic organs on dorsal aspect of mantle.)
1 species: *lucens.*
Genus *Opisthoteuthis* Verrill, 1883. (No photogenic species known.)
6 species.
Genus *Cirrothauma* Chun, 1911. (No photogenic species known.[1])
1 species.
Genus *Froekenia* Hoyle, 1904. (No photogenic species known.)
1 species.
Genus *Vampyroteuthis* Chun, 1903. (No photogenic species known.)
2 species.[2]
Genus *Lætmoteuthis* Berry, 1913. (No photogenic species known.)
1 species.

Superfamily Argonautoidea.

Family *Ocythoidæ.* (No photogenic species known.)
Genus *Ocythoe* Rafinesque, 1814.
1 recognized species.

[1] A possibility seems to exist that this genus is photogenic,—cf. Chun, : 13, p. 23–25, 27.

[2] *Cirroteuthis macrope* Berry, 1911, appears to belong to this genus.

Family *Argonautidæ*. (No photogenic species known.)
Genus *Argonauta* Linnæus, 1758.
12 ± species.
Family *Tremoctopodidæ*. (No photogenic species known.[1])
Genus *Tremoctopus* delle Chiaje, 1829.
Only 1 certainly established species.
Family *Alloposidæ*. (No photogenic species known.)
Genus *Alloposus* Verrill, 1880.
2 species.

Superfamily Amphitretoidea.

Family *Amphitretidæ*. (No photogenic species known.)
Genus *Amphitretus* Hoyle, 1885.
1 species.

Superfamily Polypodoidea.

Family BOLITÆNIDÆ.
Genus *Bolitæna* Steenstrup, 1859. (No photogenic species known.)
1 species.
Genus ELEDONELLA Verrill, 1884.
5 species: (1 species, *alberti*, described as "probablement photogene").
Genus *Vitreledonella* Joubin, 1918. (No photogenic species known.)
1 species.
Family *Polypodidæ*. (No photogenic species known.)
Genus *Polypus* Schneider, 1784.
125 ± species.
Genus *Tritaxeopus* Owen, 1881.
1 species.
Genus *Pinnoctopus* d'Orbigny, 1845.
1 species.
Genus *Scæurgus* Troschel, 1857.
4 species.
Genus *Cistopus* Gray, 1849.
2 species.
Genus *Moschites* Schneider, 1784.
3 species.
Genus *Graneledone* Joubin, 1918.
9 species.
Genus *Eledonella* de Rochebrune, 1884.
2 species.
Genus *Velodona* Chun, 1915.
1 species.

The increase afforded by the present list over the numbers included in the earlier catalogs of photogenic cephalopods is quite remarkable for the small number of years that has elapsed. Hoyle's list (:08, p. 14) records as photogenic 6 families, 26 genera, and 30 species of Œgopsida, 1 family, 3 genera and 3 species of Myopsida, and none of the other groups, or a total

[1] But cf. Tryon, '79, p. 131.

TABLE II.

RECAPITULATION OF RECENT CEPHALOPODA.

Class CEPHALOPODA.

Order TETRABRANCHIATA.

	Number of Genera.	Number of Species.	Number of Photogenic Species.
Suborder NAUTILOIDEA.			
Family *Nautilidæ*	1	3	0
	1	3	0
Order DIBRANCHIATA.			
Suborder DECAPODA.			
Œgopsida.			
Family *Architeuthidæ*	1	14	0
Family *Gonatidæ*	1	2	0
Family ONYCHOTEUTHIDÆ	6	20±	2
Family LYCOTEUTHIDÆ	2	2	2
Family LAMPADIOTEUTHIDÆ	1	1	1
Family ENOPLOTEUTHIDÆ	10	27±	27
Family OCTOPODOTEUTHIDÆ	3	3	1?
Family HISTIOTEUTHIDÆ	3	14±	14
Family BENTHOTEUTHIDÆ	2	2	2
Family *Brachioteuthidæ*	2	5	0
Family OMMASTREPHIDÆ	9	20±	2
Family *Thysanoteuthidæ*	1	2	0
Family *Lepidoteuthidæ*	1	1	0
Family CHIROTEUTHIDÆ	5	28	14
Family *Grimalditeuthidæ*	1	1	0
Family CRANCHIIDÆ	18	41	34+
	66	173±	99+
Myopsida.			
Family *Loliginidæ*	6	72±	0
Family SPIRULIDÆ	1	1+	1+
Family *Promachoteuthidæ*	1	1	0
Family *Idiosepiidæ*	1	2	0
Family SEPIOLIDÆ	12	51±	26+
Family *Sepiadariinæ*	2	3	0
Family *Sepiidæ*	4	94±	0
	27	224±	27+
Suborder OCTOPODA.			
Family CIRROTEUTHIDÆ	8	23	1
Family *Ocythoidæ*	1	1	0
Family *Tremoctopodidæ*	1	1+	0
Family *Argonautidæ*	1	12±	0
Family *Alloposidæ*	1	2	0
Family *Amphitretidæ*	1	1	0
Family BOLITÆNIDÆ	3	7	1
Family *Polypodidæ*	9	148±	0
	25	195±	2

TABLE III.

SUMMARY.

	Families.			Genera.			Species.		
	Total.	Photogenic.	Per Cent. Photogenic.	Total.	Photogenic.	Per Cent. Photogenic.	Total.	Photogenic.	Per Cent. Photogenic.
Œgopsida	16	10	62.5	66	39+	59.0	173±	99+	57.2
Myopsida	7	2	28.6	27	6+	22.2	224±	27+	11.6
Total Decapoda	23	12	52.2	93	45+	48.4	397±	126+	31.6
Octopoda	8	2	25.0	25	2	8.0	195±	2	1.0
Tetrabranchiata	1	0	0.0	1	0	0.0	3	0	0.0
Total	32	14	43.7	119	47+	39.5	595±	128+	21.3

of 7 families, 29 genera, and 33 species. Chun (:10, p. 39), treating only of the Œgopsida, increases these figures to 8 families, 26 genera and 39 species. The two sets of figures should be compared with those given in the numerical summary in Table III at the top of this page. Here it appears that of the 32 families of recent cephalopods now recognized, 14 (or more than two fifths) contain luminous species; out of 119 genera, 47 (or nearly two fifths) are light producing; and out of 595 species, 128 (or over one fifth) are now held on good ground to be luminous. The rich development in species of the genera *Loligo*, *Sepia* and *Polypus*, which has already been noted, is the circumstance chiefly responsible for the cutting down of the proportion which the luminous *species* bear to the whole to less than one half that which is exhibited by the luminous *families*. Similarly the slight proportional decline in the case of the luminous *genera* is due to the large number of ranking genera in certain mainly non-luminous families such as the Ommastrephidæ, Sepiolidæ, Cirroteuthidæ and Polypodidæ.

The table also indicates very strikingly what is really the outstanding feature of the taxonomic distribution of the photogenic forms, namely, the preponderance both of Œgopsida among the species known to be light producing, and of light producing species among the Œgopsida. In the former instance this preponderance is enormous. 71.4 per cent. of the luminous families, 83.0 per cent. of the luminous genera, 77.3 per cent. of

the luminous species, are Œgopsid. Among the Œgopsida themselves over one half of all the families, genera, and species are described as possessing photogenic organs. Five entire families—the Lycoteuthidæ, Lampadioteuthidæ, Enoploteuthidæ, Histioteuthidæ, and Benthoteuthidæ, all of them of more or less deep sea habit,—have all their species so equipped, and this seems almost certainly true of the very aberrant but numerous Cranchiidæ as well. Among other groups of cephalopods, only the Spirulidæ can aspire to inclusion in the same category, and regarding them our information is still deficient. There may be only one valid species in this family. In addition to those named, one other œgopsid family (Chiroteuthidæ) has more than half its species light producing. On the other hand luminous species for five œgopsid families (the Architeuthidæ, Gonatidæ, Thysanoteuthidæ, Lepidoteuthidæ and Grimalditeuthidæ), five of the seven myopsid families, six of the eight octopod families, and the Nautilidæ, are as yet unknown.

For multiplicity and variety of luminous forms the palm must be awarded to the Enoploteuthidæ and Cranchiidæ, though, as will subsequently appear, the maximum attainment and diversity of structure of the photogenic organs themselves is reached not in either of these families, but in the Lycoteuthidæ.

4. Actual Observance of the Phenomenon.

As compared with other Mollusca, or even with other general groups of Invertebrata, Cephalopoda, and especially those of the decapod section, are extremely difficult either to capture, to maintain alive under artificial conditions, or even to observe with any degree of satisfaction in their free condition. Among the Œgopsida it is probable that a sheer majority of the genera have never been seen at all in the living state, at any rate by any human eyes but those of fishermen. It is therefore not to be wondered at that actual observance of the phenomenon of light production in this group of animals is an extremely rare event, possible only occasionally or under very exceptional conditions. The published records of such observations are consequently so scattered that they have fallen into obscurity, or else, in the case of some of the more spectacular ones become all the more

conspicuous by very reason of their paucity and desultory character.

A brief historical survey of this subject has been given by Hoyle (:08), but the most valuable contributions thereto have been made since that time, while Hoyle himself omitted one or two quite interesting accounts from his summary. It will therefore be well to review briefly the entire field.

I have been no more successful than previous authors in the discovery of any recorded observation of photogenic phenomena in living Cephalopoda prior to that of Vérany in the case of *Histioteuthis bonnellii* (*bonelliana*), ('51, p. 119), a translation of which is quoted in full by Hoyle in the paper cited and is well worthy of repetition here.[1]

"As often as other engagements permitted, I watched the fishing carried on by the dredge on the shingly beaches which extend from the town of Nice to the mouth of the Var. On the afternoon of September 7, 1834, I arrived at the beach when the dredge had just been drawn in, and saw in the hands of a child a cuttle-fish, unfortunately greatly damaged. I was so struck by the singularity of its form and the brilliance of its color that I at once secured it, and, showing it to the fishermen, asked whether they were acquainted with it. Upon their replying in the negative I called their special attention to it, and offered a handsome reward for the next specimen secured, either alive or in good condition, and then passed on to other fishermen and repeated my promise. Shortly afterwards I was summoned and shown a specimen clinging to the net, which I seized and placed in a vessel of water. At that moment I enjoyed the astonishing spectacle of the brilliant spots, which appeared upon the skin of this animal, whose remarkable form had already impressed me: sometimes it was a ray of sapphire blue which blinded me; sometimes of opalescent topaz yellow, which rendered it still more striking; at other times these two rich colors mingled their magnificent rays. During the night these opalescent spots emitted a phosphorescent brilliance which rendered this mollusc

[1] Vérany's monograph is a very rare one in the United States, especially in the West, where I am not aware that any complete copy exists. I am accordingly entirely dependent for the information quoted upon the translation given by Hoyle.

one of the most splendid of Nature's products. Its existence was, however, of short duration, though I had placed it in a large vessel of water. Probably it lives at great depths."

Although not mentioned by Hoyle, the next student whose published observations concern us was none other than Charles Darwin. Among the melange of odd notes in the course of his account of the voyage of the "Beagle" ('60, pp. 7–8) appears the following: "I was much interested, on several occasions, by watching the habits of an Octopus, or cuttle-fish. . . . I observed that one which I kept in the cabin was slightly phosphorescent in the dark." As we have already seen, photogenic organs or tissues are practically unknown among octopods, so that this observation would be quite an anomalous and puzzling one, were it not for the at least plausible explanation that the phenomenon in this instance as so many others in the literature of biophotogenesis was due not to the active functioning of any tissues of the cephalopod itself, but to bacterial infection or even to the presence of effulgent Protozoa in the slime surrounding its skin. Another possibility which occurs to me is that the animal may not have been examined in absolute darkness, but that sufficient light penetrated into the chamber, though imperceptible to the unadjusted human eye, to enable the iridocytes in the skin of the octopus to yield a pseudo-luminous reflection, analogous to that so notorious in the case of the eyes of many mammals. The description by Giglioli of luminescent specimens of a squid which he identified as "*Loligo sagittatus*" and certain Chilean octopods, referred to by Holder in the quotation given in the next paragraph, may be susceptible of similar explanation. To the original of this work with the description of his observations I have unfortunately not been able to gain access. For my own part I have on several occasions attempted to discover similar properties in captive specimens of the common southern California devilfish, *Polypus bimaculatus* (Verrill), but so far with only negative results. Final settlement of the question can only be accomplished by careful experiment.[1]

[1] Since this paragraph was put in type I am reminded that Tryon ('79, p. 131) in his paraphrase of the description of *Tremoctopus gracilis* (Souleyet 1852) says that this species is " phosphorescent and with metallic reflections when living." I have been unable to check this observation by reference to the original work of Souleyet.

In a popular volume by C. F. Holder ('87, p. 46), descriptive of luminous organisms in general, but unfortunately none too carefully compiled, occurs the following paragraph on the Cephalopoda:

"The highest forms of the *Mollusca*, the Cephalopods, cuttle-fishes, are probably at times luminous. I have noticed what I presumed was a delicate, sensitive glow about an *Octopus* in a semi-darkened tank, but I am not satisfied to make the statement as a fact. These forms are so remarkable for the waves of color that pass over them, and which seem to make them transparent, that one could readily be deceived.

"The little *Cranchia* (Plate IV., Fig. 2) is a light-giver, its phosphorescence having been distinctly observed. It is an ally of the giant squids, which have been found fifty-five feet in length, and which, if luminous like their pygmy relative, would present a marvelous spectacle, darting veritable living arrows through the depths of the sea.

"Giglioli refers to the phosphorescence of *Loligo sagittatus*, and to that of several small Octopods observed by him at Callao and Valparaiso. Their bodies gave out a pale whitish light, uniformly distributed."

It happens that *Cranchia* is a genus which is now known to possess definite photogenic organs, but these have been found to occur only on the eyeball, whereas the rather poor figure given by Holder represents the animal as brightly and evenly glowing over the entire surface,—body, head, arms, tentacles, and all. As to the supposed photogenic properties of *Polypus* and related octopods,—both Darwin and Giglioli would seem to have been too accurate observers for the explanation advanced by Holder to be entirely satisfactory.

Chun, in his narrative of the cruise of the "Valdivia" (:03, pp. 569–570; also :03*a*, p. 81; :10, p. 50) gives up to this time the fullest account of the actual display of photogenic propensity by a cephalopod we have been able to find, and he followed this in later publications by a very considerable contribution to our morphological knowledge of the organs responsible for the manifestation. The specimen observed proved to belong to a wonderful undescribed species, the *Lycoteuthis diadema* (Chun).

Although it was taken from a considerable depth, he was able to keep it alive in ice water long enough to make a photograph of it by dint of its own light. Again I must quote from a translation by Hoyle: "Among all the marvels of coloration which the animals of the deep sea exhibited to us, nothing can be even distantly compared with the hues of these organs. One would think that the body was adorned with a diadem of brilliant gems. The middle organs of the eyes shone with ultramarine blue, the lateral ones with a pearly sheen. Those towards the front of the lower surface of the body gave out a ruby-red light, while those behind were snow-white or pearly, except the median one, which was sky-blue. It was indeed a glorious spectacle." It is altogether a pity that similar observations have not been possible for the doubtless even more spectacular *Nematolampas regalis*, which, although very nearly related to *Lycoteuthis* is equipped with an entire further battery of photophores.

More detailed from the standpoint of physiology is the account given by Watasé (:05) of a little squid, the "hotaru-ika" of Japanese writers,[1] which is extremely abundant at the proper season and locality on certain of the shores of Japan, and which has since become the best known of all the luminous squids. Watasé's paper is an important one as the first dealing with this species, but, being semi-popular in character and published in Japanese, escaped notice for a considerable time and has only lately received a little of the attention it deserves. Through the kindness of Mr. Sotaro Matsushita, formerly of Redlands, California, I have for some time been in possession of a translation, and a very free transcription of some of its more interesting if quaint passages should be neither inappropriate nor unwelcome here. "Hotaru-ika, when seen externally, does not differ much from other *ika* [squids]. Yet there are many interesting features which we do not see in other *ika*. At each end of the two 'legs' there are three oblong, black spots. These small spots were first discovered by the French scientist Joubin. Yet even he did not know their function. According to the results of my own study of these in living Japanese specimens, the spots were found to produce a considerable light, penetrating to the space of about

[1] The "firefly squid"—*Watasenia scintillans* (Berry).

a foot. . . . While the animal is living these spots are transparent.

"Again there are hundreds of other small spots all over the body. . . . When seen in daylight they appear to be small black spots, but in the night all these spots shine with a brilliant light like that of the stars in heaven. . . . When these spots (while the *hotaru-ika* is alive) are viewed under the microscope, they are very interesting. When the animal is about to produce the light, the membranes [chromatophores] covering the spots will concentrate and remove themselves, thus opening a way for the light. The light is so brilliant that it seems like a sunbeam shot through a tiny hole in a window curtain. Again when the *hotaru-ika* wishes to shut off the light, the membranes will expand and cover the spots. . . ."

In the following year Meyer (:06) described briefly the photogenic activity of the myopsid, *Heteroteuthis dispar* (Rüppell), similar observations having been made some time previously by Lo Bianco, but never published. Meyer found that in the case of the specimen observed by him at the Naples Zoölogical Station he "could in the dark room easily locate the position of the photophore through the transparent mantle, lying on the ventral surface just behind the anus." He further found that when the animal was irritated, "it shot rapidly through the water, and spurted through its funnel a luminous secretion which floated in the water as separate globules, these being drawn out by the currents into shining threads, a pyrotechnic display (*Feuerwerk*) which he was able to repeat many times. The light of the secretion and of the light organ itself had the same pale greenish hue which we observe with our glow-worms." Meyer further reports the discovery by one of his colleagues, Marchand, of somewhat similar photogenic properties in *Sepiola*, except that in this genus "the luminous secretion is not discharged into the water but remains on the surface of the gland. Furthermore, *Sepiola* only shines if it be very powerfully stimulated, as when, for instance, the mantle is cut open." From the foregoing it is at once evident that in both these myopsid genera the mechanism of light production is very different from that of any of the other forms studied, and this conclusion is borne out by the anatomical

features. The possible utility of this peculiar development of the function, so far as *Heteroteuthis* is concerned, is the subject of some interesting speculation in one of Meyer's subsequent papers (:08, pp. 507–508), which will receive more attention later on.

We are now brought to a consideration of some important recent work performed by various Japanese observers in continuation of Watasés pioneer studies on *Watasenia scintillans* (Sasaki, :12, :13, :14; Ishikawa, :13). This constitutes probably the chief work which has been done in this field, and therefore merits consideration in considerable detail.

"In the region where the squids live, that is, in the waters of Namerikawa on the coast of the Japan Sea," writes Ishikawa (:13, pp. 167–169), "this circumstance [the luminosity of the tips of the ventral arms described by Watasé] had already long been known; but none of our zoölogists were aware of it until Watasé by chance made the discovery. At a time when he was engaged in the study of fireflies, he was apprised by a schoolmaster that there occurred a species of squid in the sea at Namerikawa which lighted very strongly. Pursuant to this suggestion he sought the village named, found in due course a species of small squid with powerful light organs, and recognized the same as a species of *Abraliopsis*. As he has orally told me, it was on the 28th of May, 1905, on the memorable day of the battle of Tsusima, hat he saw the light of this squid for the first time. . . . . .

"The large swellings at the tips of the ventral arms, as well as two or three smaller dots, are, as he remarks, luminous organs of the first order. They shine so brilliantly that when one observes the animals in dark water, one sees only two effulgent bodies moving in the dark water, like the glow of an electric contact, and the lively oscillations of the invisible arms produce a very wierd effect. Next to these in the intensity of their light are the eye organs, and then come the remaining organs. The three types of organs do not always shine simultaneously; often only one or the other. But it can also happen that the animal sets all the organs into action at the same time. When the mantle organs light up, the form of the animal springs out spectre-like in the dark water. These organs, arranged in rows, when one

examines them close at hand, shine like an electric illumination. The color of the light is a beautiful clear blue.

"As Watasé writes, the arm organs in dead animals are entirely surrounded by pigment cloaks and only when alive can the animal retract these. The retraction of these cloaks takes place very quickly, and when they are retracted, the organ appears in daylight as a delicate dull-green colored body."

In a paper which comprises a most notable contribution to our knowledge of the ecology and habits of ten-armed cephalopods, Sasaki (:14, pp. 77–80) adds materially to the accounts of his predecessors. Some of his observations are so pertinent to some of the discussion which must follow later that they should be quoted rather fully. Treating the three types of photophore to be seen in *Watasenia* under separate headings, this author writes:

"*Brachial Organ.* This is the largest organ, and when I made observations in the fishing season, it was much more active in phosphorescence than other organs. It is situated at the end of each ventral arm, composed of 3 globules arranged in a series. The globules are ovoid in shape and nearly equal in size, but the middle one in the series is generally a little larger than the others, the dimensions being 1.4 mm. long and about 1 mm. broad. In fresh specimens they show a greenish cobalt colour, and there are 2 or 3 layers of large brownish chromatophores covering a part of the preceding substance. These chromatophores are constantly contracting and expanding. When they were observed at night on the living animals, they were seen to discharge light in all directions much brighter than any of Japanese fireflies. The color of the light is Prussian-blue or tinged a little with purple, and the luminosity is strong enough to outshine the other luminous organs. When the living animal was placed on a glass plate, which was put directly on the case of the dry plate of the photographic camera, and then exposed for four seconds with the Lion's dry plate of the special rapid no. 230, the light of this brachial organ was distinctly taken on the dry plate, although those of other organs made no impression.

"*Minute Organs Scattered on the Ventral Surface of the Whole Body.* There are numerous minute organs distributed on the

ventral surface of the mantle, head and siphon, and they are also on the third and fourth arms. . . .

"Each organ in the fresh specimen has a substance of purplish hue in the centre; this substance seems to be that discharging light when the animal is living. When the organ is exposed in the air, the purplish hue of the substance changes to greenish blue after a while, and finally resolves into a true green. The substance is covered by a pigment layer of darkish brown or deep purple which has a hole resembling the pupil of an eye, through which the substance can easily be seen. The light of the substance at night is whiter and less luminous than that of the brachial organ.

"*Ocular Organ.* When the eyelid of the fresh specimen is removed and the eyeball exposed, there are seen 5 luminous organs arranged in a series along the ventral circumference of the eyeball, the organ on either end of the series being a little larger than the remaining 3. The colour of all these organs is pearly white. When the organ is seen at night in the living animal, the phosphorescence is not distinguishable from that of the minute organ on the body.[1]

"*Difference of Phosphorescence in the Sexes.* On examining the preserved specimens to discover the difference of the external forms as well as the histological structures of their luminous organs as occurring in the male and female, none could be discerned. But in the female specimens there are one hundred or so more of the minute organs of the mantle than in the male. Whether there is any meaning as to sexual selection, it is difficult to say, the data concerned being insufficient at present to announce any opinion.

"Next, as to the difference of phosphorescence between the sexes in their living state, the means of investigation proved to be very difficult. At first I repeatedly undertook to keep the animal in an aquarium, but no success was attained. The reason for the failure is that first of all, the animals are very delicate, and next the aquarium was defective. The animals

[1] A slight discrepancy is noteworthy between the account of Sasaki and that of Ishikawa concerning the character of the light of the subocular organs. According to the latter these photophores are more or less intermediate in brilliancy between the large brachial organs and those of the general integument.

are so weak that in carrying them from the sea to the aquarium they wasted and died. As they wasted, the luminosity in question became very feeble, and naturally with their expiration, the light of the luminous organs gradually vanished altogether. This being so, I then tried to observe the animals directly while they were swimming in the net. But no good means were found easily to distinguish the sexes on such dark nights, even with the feeble light of the moon or of a lantern.

"However in my examinations at night, no special variety of the light could be found, the colour of the light being always the same. And in one case, putting in a vessel and observing about thirty specimens in a fishing boat while they were yet actively on motion, I verified the fact that their luminosity is uniform. In the morning, to my surprise, a male was found dead among those 30 specimens; this proves that it had the same colour of light with the female on that night. The above data seem to prove the fact that the colour of the light of the luminous organs is the same in both sexes.

"Again, in late July of the same year, I made another observation on the phosphorescence under consideration and then it was quite evident to me that the luminosity of the brachial organ was at this season noticeably feebler than in the spring.

"The phosphorescence of the immature animal can never be studied in Namerikawa, young ones thus far not being found there."

In the same paper (pp. 98–99), Sasaki incidentally records the fact that he observed the photogenic property in living specimens of the myopsid, *Inioteuthis japonica* Verrill (= *inioteuthis* (Naef)). These he found to be "discharging a faint cobaltish light from a great luminous organ which is situated in the mantle cavity near the ink-bag." From anatomical observations we know that the luminous organs of this genus are essentially similar to those of the nearly related if not actually congeneric *Sepiola*.

Lastly, Dahlgren (:16, pp. 70–71) describes in a little greater detail than before the photogenic behavior of *Heteroteuthis dispar*, the myopsid species already observed by Meyer. He writes: "When brought into the laboratory in good condition

and allowed to rest quietly it may be taken into the dark-room and gently struck, as it swims in the aquarium, with a glass rod. Fig. 18 is a drawing to illustrate what may and usually does happen under these circumstances. The animal throws out of its siphon several little masses of mucus which show no light at the moment of ejection, but almost instantly, as the oxygen of the water begins to work on them, show a number of rod-shaped particles of a brilliantly luminous matter embedded throughout the very delicate mass. As the mass continues to expand this light continues to glow brightly for as much as three to five minutes, after which it rather suddenly dies out. In color the light is the usual blue-green of luciferine when burning outside the body. The animal can repeat this process for a number of times, when it appears to have exhausted its supply of luciferine, and it is not possible, apparently, to keep it in captivity for a long enough period for the supply to be restored."

From this scanty, but for all practical purposes probably exhaustive summary, we find that except for the doubtful observations by Souleyet, Darwin and Giglioli, the actual process of light production in cephalopods has been observed directly in but seven species, of which three belong to the myopsid family Sepiolidæ and have photogenic organs of a peculiar discharging type, while the other four belong to the Œgopsida. We are fortunate, however, in that each of these latter species is representative of a different family and thus ample support is given to the inferences necessarily drawn from the outward appearance and histological structure of the many types of photophore that they are of a fact photogenic.

(To be Continued)